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(54) **COMPLEMENT FACTOR-I FORMULATIONS**

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(71) Applicant: **Vertex Pharmaceuticals Incorporated**,
Boston, MA (US)

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(72) Inventors: **Bing SHAN**, San Jose, CA (US); **Brian David CONNOLLY**, Berkeley, CA (US); **Sibu BALAN**, Redwood City, CA (US); **Anju CHATTERJI**, Martinez, CA (US)

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(57) **ABSTRACT**

Provided herein are pharmaceutically acceptable formulations comprising wild type Complement Factor I (CFI) and variants thereof; in some embodiments the wild type CFI and variants thereof are part of a fusion construct, e.g. a fusion construct comprising human serum albumin. The formulations stabilize CFI against acute stresses during storage in either a liquid or lyophilized state. Also provided are methods of making the formulations, and methods of using the formulations in the treatment of diseases.

Specification includes a Sequence Listing.

COMPLEMENT FACTOR-I FORMULATIONS

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. Provisional Application No. 63/293,013 filed on Dec. 22, 2021, the contents of which are incorporated herein by reference in their entirety.

REFERENCE TO AN ELECTRONIC SEQUENCE LISTING

[0002] The contents of the electronic sequence listing (VTEX_707_01WO_SeqList_ST26.xml; Size: 31,757 bytes; and Date of Creation: Dec. 17, 2022) are herein incorporated by reference in its entirety.

BACKGROUND

[0003] Complement Factor I (CFI) is a soluble protein of the complement system, that regulates complement activation by cleaving cell-bound or fluid phase C3b and C4b. It is a soluble glycoprotein that circulates in human blood and acts to maintain the balance between the classical, lectin, and alternative pathways of the complement system. Dysregulated CFI, mutated and dysfunctional CFI, and CFI deficiency have been implicated in diseases involving the complement system. Needed are pharmaceutically acceptable CFI formulations useful for regulating complement system. Provided here are formulations address this need.

SUMMARY

[0004] The disclosure provides formulations comprising wild type complement factor I (CFI) or CFI variants, and fusion proteins thereof. The formulations provided stabilize the active ingredient against acute stresses and allow for storage in both liquid and lyophilized states. Also provided are methods of making the formulations, and methods of using the formulations in the treatment of diseases.

DETAILED DESCRIPTION

[0005] The disclosure provides formulations comprising wild type CFI or CFI variants, and fusion proteins thereof. The formulations provided stabilize the active ingredient against acute stresses and allow for storage in both liquid and lyophilized states. Also provided are methods of making the formulations, and methods of using the formulations in the treatment of diseases.

I. Pharmaceutically Acceptable Formulations

[0006] The pharmaceutically acceptable formulations described herein provide stabilizing properties to wild type CFI or CFI variants, and fusion constructs thereof of the disclosure (interchangeably referred to herein here as the “active ingredient” or “active pharmaceutical ingredient”) at a range of concentrations that allow for pharmaceutically acceptable storage conditions. Stabilizing properties can be, for example, prevention of degradation, maintenance of concentration, prevention of aggregation, and/or maintenance of bioactivity.

[0007] The formulations of the disclosure include wild type CFI, CFI variants, fusion constructs comprising wild type CFI (e.g. CFI-HSA), or fusion constructs comprising a CFI variants (e.g. any CFI variant of Table 2-HSA fusion),

buffering agents, tonicity modifiers, surfactants, and further optionally bulking agents, cryoprotectants, lyoprotectants, and/or stabilizers.

[0008] The formulation can be designed to support storage of the active ingredient as a solid (dry) form (e.g. lyophilized cake or cryopreserved). In some embodiments, the formulation is a lyophilizate. In some embodiments, the formulation is a liquid formulation.

[0009] The formulations can be designed to support storage (while maintaining, e.g. stability, activity) of the active ingredient in solution as a liquid at a range of concentrations. The concentration can be adjusted in the formulation for use in different types of administration (e.g. subcutaneous or intravenous). For example, the active ingredient can be stored in the formulation as a liquid solution at about 10 mg/mL to about 300 mg/mL, for example at about 10 mg/mL, about 15 mg/mL, 20 mg/mL, about 25 mg/mL, 30 mg/mL, about 35 mg/mL, 40 mg/mL, about 45 mg/mL, about 50 mg/mL, about 55 mg/mL, about 60 mg/mL, about 65 mg/mL, about 70 mg/mL, about 75 mg/mL, about 80 mg/mL, about 85 mg/mL, about 90 mg/mL, about 95 mg/mL, about 100 mg/mL, about 105 mg/mL, about 110 mg/mL, about 115 mg/mL, about 120 mg/mL, about 125 mg/mL, about 130 mg/mL, about 135 mg/mL, about 140 mg/mL, about 145 mg/mL, about 150 mg/mL, about 155 mg/mL, about 160 mg/mL, about 165 mg/mL, about 175 mg/mL, about 180 mg/mL, about 185 mg/mL, about 190 mg/mL, about 195 mg/mL, about 200 mg/mL, about 205 mg/mL, about 210 mg/mL, about 215 mg/mL, about 220 mg/mL, about 225 mg/mL, about 230 mg/mL, about 235 mg/mL, about 245 mg/mL, at about 250 mg/mL, at about 255 mg/mL, at about 260 mg/mL, at about 260 mg/mL, at about 265 mg/mL, at about 270 mg/mL, at about 275 mg/mL, at about 280 mg/mL, at about 285 mg/mL, at about 290 mg/mL, at about 295 mg/mL, or about 300 mg/mL. In some embodiments, the formulation comprises the active ingredient at a concentration of about 50 mg/mL. In some embodiments, the formulation comprises the active ingredient at a concentration of about 100 mg/mL. In some embodiments, the formulation comprises the active ingredient at a concentration greater than 150 mg/mL. In some embodiments, the formulation comprises the active ingredient at a concentration of about 170 mg/mL. In some embodiments, the formulation comprises the active ingredient at a concentration of about 190 mg/mL.

[0010] The formulations of the disclosure allow the active ingredient to maintain stability (e.g. activity) at any one or more of the following temperatures: $-80^{\circ}\text{C.}/-2^{\circ}\text{C.}$, $-20^{\circ}\text{C.}/-2^{\circ}\text{C.}$, $0^{\circ}\text{C.}/-2^{\circ}\text{C.}$, $4^{\circ}\text{C.}/-2^{\circ}\text{C.}$, $25^{\circ}\text{C.}/-2^{\circ}\text{C.}$, $37^{\circ}\text{C.}/-2^{\circ}\text{C.}$, $45^{\circ}\text{C.}/-2^{\circ}\text{C.}$, or at $60^{\circ}\text{C.}/-2^{\circ}\text{C.}$

[0011] The formulations of the disclosure allow the active ingredient to maintain stability (e.g. activity) for at least 1 week, at least 2 weeks, at least 3 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, at least one year, at least 2 years, or more.

[0012] The pH of the formulation described herein is any pH that provides stabilizing properties to the CFI variants and fusion constructs described herein. In some embodiments, the formulation comprises a pH between about 5 to about 7.5. The pH can be, for example, about pH 5.0, about pH 5.1, about pH 5.2, about pH 5.3, about pH 5.4, about pH 5.5, about pH 5.6, about pH 5.7, about pH of 5.8, about pH

5.9, about pH 6.0, about pH 6.1, about pH 6.2, about pH 6.2, about pH 6.3, about pH 6.4, about pH 6.5, about pH 6.6, about pH 6.7, about pH 6.8, about pH 6.9, about pH 7, about pH 7.1, about pH 7.2, about pH 7.3, about pH 7.4, or about pH 7.5.

[0013] The formulations include a pharmaceutically acceptable buffering agent. Buffer agents can be used to maintain the pH of the formulation in a desired range. Any suitable buffer can be used in the formulations of the disclosure. Examples of pharmaceutically acceptable buffers comprise histidine, acetate, citrate, succinate, tartrate, glutamate, glycine, bicarbonate, sulfate, nitrate, phosphate, and hydroxymethylaminomethane (tris) buffers. In some embodiments the buffering agent is present at about 1 mM to about 50 mM. In some embodiments the buffering agent is present at about 20 mM. In some embodiments, the buffer comprises histidine, e.g. histidine hydrochloride. In some embodiments, the buffer comprises acetate, e.g. sodium acetate. In some embodiments, the buffer comprises succinate, e.g. sodium succinate.

[0014] The formulations include a surfactant, sometimes referred to as a wetting and/or solubilizing agent. Without limitation, a surfactant excipient can be used to modulate solubility and bioavailability of biologic molecules, increasing the stability of biologics molecules in dosage forms, and maintain preferred polymorphic forms. Examples of surfactants include polysorbate 20 (PS20, Tween 20), polysorbate 80 (PS80, Tween 80), PS80/20, poloxamer (Pluronic F68 and F127), Triton X-100, Brij 30, and Brij 35. In some embodiments, the formulation of the disclosure comprises a surfactant. In some embodiments, the surfactant is polysorbate 20 or polysorbate 80. In some embodiments, the formulation comprises polysorbate 20. In some embodiments, the polysorbate 20 is about 0.01% to about 0.05%. In some embodiments, the polysorbate is about 0.02%. In some embodiments, the formulation comprises polysorbate 80. In some embodiments, the polysorbate 80 is about 0.01% to about 0.05%. In some embodiments, the polysorbate 80 is about 0.02%.

[0015] The formulations include a tonicity modifier, sometimes may also be referred to as a tonicity agent, tonicifier or tonicifying agent. Tonicity modifiers are used to adjust the osmolality of the formulation such that the osmotic pressure of the formulation when administered to a subject does not cause any deleterious effects, such as unwanted cell lysis. Any pharmaceutically acceptable tonicity modifiers that is compatible with the active ingredients of the disclosure may be suitable for use in the formulations described herein. Pharmaceutically acceptable tonicity modifiers can include, for example, mannitol, sorbitol, lactose, dextrose, trehalose, sodium chloride, potassium chloride, glycerol, glycerin, or arginine hydrochloride. In some embodiments, the formulation comprises a tonicity modifier. In some embodiments, the tonicity modifier is arginine hydrochloride. In some embodiments, the formulation comprises arginine hydrochloride. In some embodiments, the arginine hydrochloride is about 10 mM to about 200 mM. In some embodiments, the arginine hydrochloride is about 70 mM. In some embodiments, the arginine hydrochloride is about 135 mM. In some embodiments sorbitol is the tonicity modifier, and the sorbitol is present at about 1% to about 10% v/v, e.g. at about 5% v/v. In some embodiments the tonicity modifier is trehalose, and trehalose is present at about 1% to about 15% v/v, e.g. at about 10% v/v. In some

embodiments the tonicity modifier is sodium chloride, and is present at about 1 mM to about 500 mM, e.g. at about 150 mM. In some embodiments the tonicity modifier is sorbitol or trehalose for non-ionic buffering agents, and sodium chloride for ionic buffering agents.

[0016] The formulation can include a bulking agent, for example a trehalose or glycine. In some embodiments, the bulking agent is glycine and is present in the formulation at about 1% to about 5% v/v, e.g. at about 2%. In some embodiments, the bulking agent is glycine and is present in the formulation at about 1 mM to about 100 mM, e.g. at about 60 mM.

[0017] The formulation can include a cryoprotectant and/or a lyoprotectant. Cryoprotectants and lyoprotectants are included in formulations to provide protection to biologic molecules from denaturation during cycles of freezing and thawing. They can also aid in preserving biologic integrity, e.g. maintaining bioactivity, during lyophilization (i.e. freeze drying) and reconstitution. In some instances, cryoprotectants and lyoprotectants can be referred to interchangeably. Any pharmaceutically acceptable cryoprotectant and/or lyoprotectant capable of providing stabilizing properties to formulations including the CFI variants and fusion constructs of the disclosure can be used. Non-limiting examples of cryoprotectants and lyoprotectants include polyols, disaccharides, polysaccharides, glucose, glycine, mannitol, sorbitol, sucrose, trehalose, and dextran 40. In some embodiments, the formulation comprises a cryoprotectant. In some embodiments, the cryoprotectant is at least one of sucrose, glycine, sorbitol, trehalose, and mannitol. In some embodiments, the cryoprotectant is sucrose. In some embodiments, the sucrose is about 2% to about 10%. In some embodiments, the sucrose is about 4% to about 5%. In some embodiments, the sucrose is about 8.5%. In some embodiments, the cryoprotectant is glycine. In some embodiments, the glycine is about 50 mM to about 150 mM, e.g. about 120 mM. In some embodiments, the glycine is about 120 mM. In some embodiments, the cryoprotectant is sorbitol. In some embodiments, the glucose is present in the formulation at about 1% to about 10% v/v, e.g. at about 4% v/v. In some embodiments, the sorbitol is present at about 1% to about 5%. In some embodiments, the sorbitol is about 2.5% v/v. In some embodiments, the trehalose is present at about 1% to about 10% v/v, e.g. about 4% v/v. In some embodiments, the sorbitol is about 2.5%. In some embodiments, the cryoprotectant is mannitol. In some embodiments, the mannitol is about 1 mM to about 100 mM. In some embodiments, the mannitol is about 60 mM.

[0018] The formulation can include a stabilizing agent, sometimes referred to as a stabilizer. A stabilizing agent is any excipient that provides stabilizing properties to formulations that include the CFI variants and fusion constructs described herein. For example, a stabilizing agent can be calcium chloride (CaCl_2), histamine, methionine, ascorbic acid, glutathione, vitamin E, poly(ethylenimine), chelating agents, antimicrobial preservatives, antioxidant preservatives, or any other pharmaceutically acceptable stabilizing agent. In some embodiments the formulation comprises a stabilizer. In some embodiments, the stabilizer is calcium chloride. In some embodiments, the formulation comprises calcium chloride. In some embodiments, the calcium chloride is about 20 mM to about 50 mM. In some embodiments, the calcium chloride is about 35 mM.

a CFI base molecule is a wild type, non-human primate CFI. In some embodiments, a CFI base molecule comprises only some domains of a wild type, human CFI.

[0046] In some embodiments, the CFI variants provided herein modulate the activity of the complement system and have at least one improved characteristic as compared to a wild type CFI. Such improved characteristics include, but are not limited to an increase or decrease in any one or more of bioavailability, half-life, activity, potency, catalytic capability, cofactor affinity (e.g. affinity for Factor H and/or CR1), substrate specificity and substrate affinity (e.g. affinity for C3b and/or C4b). In some embodiments, the improved characteristic is increased half-life. In some embodiments, the improved characteristic is an increase in activity, discussed further in detail, in subsequent sections below. In

other embodiments, the improved characteristic is a change in substrate specificity for C3b and/or C4b, allowing for tunability of the CFI variant.

[0047] Provided in Table 1 are exemplary base molecules that may be used for the generation of CFI variants. The base molecules provided herein may be useful for modulation of the complement system without further modification, or may be useful for modulation of the complement system with further modification. For example, any one of the base molecules provided in Table 1 may be further modified to include one or more modifications, such as a deletion of one or more amino acid residues, a deletion of one or more CFI domains, a substitution of one or more amino acid residues, or an addition of one or more amino acid residues or CFI domains. The base molecules of Table 1 may be further part of a fusion construct, further described below.

TABLE 1

Base Molecules for Generation of CFI Variants		
Description of CFI Base Molecule	Nomenclature of Base Molecule	Amino Acid Sequence
Plasma derived, wild type human CFI (wt hCFI) (with signal sequence underlined)	CFI-PD	MKLLHVFLLFLCFHLRFCKVITYTSQEDLVEKKCLAKKYTHLSCDKVFCQPWQRCIEGTCVCKLPYQCPKNGTAVCATNRRSFPTYCQQKSLECLHPGTKFLNNGTCTAEGKFSVSLKHGNTDSEGIVEVKLVLDQDKTMFICKSSWSMREANVACLDLGFQGGADTQRRFKLSLDLSINSTECLHVHCRGLETSLAECTFTKRRTMGYQDFADVVCYTQKADSPMDDFQCVNGKYISQMKACDGINDCGDSDELCKKACQKGFHCKSGVCIPSQYQCNGEVDCTITGEDEVGCAGFASVTQEETEIILTADMDAERRRIKSLLPKLSGKVRNRMHIRRKRIVGGKRAQLGDLPWQVAIKDASGITCGGIYIGGCWILTAACHLRASKTHRYQIWTTVVDWIHPDLKRIVIEYVDRIIFHENYNAGTYQNDIALIEMKKGDKNKKDCELPRSIPACVPWSPYLFQPNPNTCIVSGWGREKDNERVFSLQWGEVKLISNCSKFYGNRFYEKEMECAGTYDGSIDACKGDSGGPLVCMDDANNVTYVWGVVSWGKPEFPFGVYTKVANYFDWISYHVGRRPFISQYNV (SEQ ID NO: 1)
wt hCFI, no signal sequence	hCFI	KVITYTSQEDLVEKKCLAKKYTHLSCDKVFCQPWQRCIEGTCVCKLPYQCPKNGTAVCATNRRSFPTYCQQKSLECLHPGTKFLNNGTCTAEGKFSVSLKHGNTDSEGIVEVKLVLDQDKTMFICKSSWSMREANVACLDLGFQGGADTQRRFKLSLDLSINSTECLHVHCRGLETSLAECTFTKRRTMGYQDFADVVCYTQKADSPMDDFQCVNGKYISQMKACDGINDCGDSDELCKKACQKGFHCKSGVCIPSQYQCNGEVDCTITGEDEVGCAGFASVTQEETEIILTADMDAERRRIKSLLPKLSGKVRNRMHIRRKRIVGGKRAQLGDLPWQVAIKDASGITCGGIYIGGCWILTAACHLRASKTHRYQIWTTVVDWIHPDLKRIVIEYVDRIIFHENYNAGTYQNDIALIEMKKGDKNKKDCELPRSIPACVPWSPYLFQPNPNTCIVSGWGREKDNERVFSLQWGEVKLISNCSKFYGNRFYEKEMECAGTYDGSIDACKGDSGGPLVCMDDANNVTYVWGVVSWGKPEFPFGVYTKVANYFDWISYHVGRRPFISQYNV (SEQ ID NO: 5)
Δ(K1-P305), deletion of A-chain of wt hCFI	ΔA-chain (CFI-SPD)	KLSGKVRNRMHIRRKRIVGGKRAQLGDLPWQVAIKDASGITCGGIYIGGCWILTAACHLRASKTHRYQIWTTVVDWIHPDLKRIVIEYVDRIIFHENYNAGTYQNDIALIEMKKGDKNKKDCELPRSIPACVPWSPYLFQPNPNTCIVSGWGREKDNERVFSLQWGEVKLISNCSKFYGNRFYEKEMECAGTYDGSIDACKGDSGGPLVCMDDANNVTYVWGVVSWGKPEFPFGVYTKVANYFDWISYHVGRRPFISQYNV (SEQ ID NO: 12)

TABLE 1-continued

Base Molecules for Generation of CFI Variants		
Description of CFI Base Molecule	Nomenclature of Base Molecule	Amino Acid Sequence
Wild type mouse CFI (https://www.uniprot.org/uniprot/Q61129)	Mouse CFI (mCFI)	MKLAHLSLFLALHLSSSRSPSASDLPQEELVDQK CLLQKYTHRSCNKVFCQPWQRCIEGTCICKLPYQ CPRAGTPVCAMNGRSYPTYCHQKSFECLEHPEIKFS HNGTCAAEKGFNVSLIYGRKTEGLVQVKLVDQ DERMFICKNSWSMAEANVACVDLGFPLGVVDIQ GSFNISGNLHINDTECLHVHCRGVETSLAECFTK RRTELSNGLAGVVCYKQDADFPTSLSFQCVNGKH IPQEKACGNVNDGQSDCLCKGCRGNASLCKS GVCIPDQYKCNGEVDCITGEDESRCEDRQQNIPK GLARSAQGEAEIETEEMLTGMDNERKRIKSL PKLSCGVKRNTHTRRKRVIGGKPANVGDPYQWQ AIKDGQRITCGGIYIGGCWILTAACHVRPSRAHSY QVWTALLDWLKPNSQLGIQTVKRIVVHEKYNGA TFQNDIALIEMKMHTGKKECELPNSVPACVPWSP YLFQPNDRCIISGWGRGKDNQKVYSLRWGEVDLI GNCSQFYDPDRYEEKEMQCAGTRDGSIDACKGDS GGPLVCEDINNVTYVVGIVSWGECGKPEFPGVY TRVANYFDWISYHVGRSLVSQHN (SEQ ID NO: 23)
wt hCFI + GSSGG (linker) + wt hCFI	hCFI-hCFI fusion	KVITYTSQEDLVEKKCLAKKYTHLSCKVFCQPW QRCIEGTCVCKLPYQCPKNGTAVCATNRRSFPTY CQQKSLCCLHPGTFKLNNGTCTAEGKFSVSLKHG NTDSEGIQVVKLVQDKTMFICKSSWSMREANVA CLDLGFQGGADTQRRFKLSLSDLSINSTECLHVHCR GLETSLAECTFTKRRTMGYQDFADVVCTQKAD SPMDDFFQCVNGKYISQMKACDGINDCGDQSDCL CKACQGGKGFHCKSGVCIPSYQCNGEVDCITGE DEVGCAGFASVTQEETEILTADMDAERRRIKSLLP KLSCGVKNRMHIRRKRIVGGKRAQLGDLPWQVA IKDASGITCGGIYIGGCWILTAACHLRASKTHRYQI WTTVVDWIHPDLKRIVIEYVDRIIFHENYNAGTYQ NDIALIEMKKDGNKKDCELPRIIPACVPWSPYLFQ PNDTCIVSGWGREKDNERVFSLQWGEVKLISNCS KFYGNRFYEEKEMECAGTYDGSIDACKGDSGGPL VCMDDNNVTYVVGIVSWGECGKPEFPGVYTK VANYFDWISYHVGRPFISQYNVSGSGGKVITYTSQ EDLVEKKCLAKKYTHLSCKVFCQPWQRCIEGTC VCKLPYQCPKNGTAVCATNRRSFPTYCQQKSLC LHPGTFKLNNGTCTAEGKFSVSLKHGNTDSEGIQ VKLVQDKTMFICKSSWSMREANVACLDLGFQ GADTQRRFKLSLSDLSINSTECLHVHCRGLETSLAEC TFTKRRTMGYQDFADVVCTQKADSPMDDFFQ VNGKYISQMKACDGINDCGDQSDCLCKACQGGK GFHCKSGVCIPSYQCNGEVDCITGEDEVGCAGF ASVTQEETEILTADMDAERRRIKSLLPKLSCGVKN RMHIRRKRIVGGKRAQLGDLPWQVAIKDASGITC GGIYIGGCWILTAACHLRASKTHRYQIWTTVVDW IHPDLKRIVIEYVDRIIFHENYNAGTYQNDIALIEM KKDGNKKDCELPRIIPACVPWSPYLFQPNPTCIVS GWGREKDNERVFSLQWGEVKLISNCSKFYGNRF YEKEMECAGTYDGSIDACKGDSGGPLVCMDDNN VTYVVGIVSWGECGKPEFPGVYTKVANYFDW ISYHVGRPFISQYNV (SEQ ID NO: 16)

[0048] In some embodiments, a base molecule itself may be a CFI variant, for example in some embodiments, a CFI variant comprising only the serine protease domain (CFI-SPD) itself is a CFI variant. In some embodiments, the CFI variants are derived from any base molecule of Table 1, and comprise modifications to loops corresponding to the loops of an unmodified CFI. In some embodiments, the CFI variants are derived from any base molecule of Table 1, and comprise substitution mutations. In some embodiments, the CFI variants are derived from any base molecule of Table 1, and comprise a deletion of one or more domains of CFI. In some embodiments, the CFI variants are derived from any

base molecule of Table 1, and comprise an inversion of the A-chain and the B-chain of the CFI.

[0049] In some embodiments, provided herein are CFI variants comprising at least one CFI domain, wherein the at least one CFI domain corresponds to those of a wild type CFI of any species. For example, the amino acid sequence of the at least one CFI domain may comprise the amino acid sequence derived from a wild type human CFI as set forth in SEQ ID NO: 5. The CFI variants provided herein comprising an amino acid sequence derived from SEQ ID NO: 5 may comprise one or more modifications with respect to

TABLE 2

Exemplary CFI Variants and CFI Variant Combinations		
Variant Description	Variant Description	Variant Description
K14A	AC-term (Δ558-	N531A
Y20A	PFISQYNV-565	P535A
Y20F	(SEQ ID NO: 25))	Y408F
D26A	R557A	Y408F; N531G
F29A	K326A; R327A	Y408L; N531G; E457G;
R35A	Y408L; N531G	E461Q; R462K; F464Y
E38A	L307G	E530D
M220A; K221Q	N531G; P535A	E457G
S507A	Y408L	E461Q
S250A	456-REKDNERVFS-465	R462K
S250L	(SEQ ID NO: 26) →	F464Y
Δ(K1-P305)	NTASSGADYPDE	I317D; R318D; R319D;
D425A	(SEQ ID NO: 27)	K320D; R321K
D425K	E457G; E461Q; R462K;	Δ(K1-P305); N531G
D425R	F464Y	Δ(K1-P305); Y408L;
514-MDANNVT-520	E38A; D425R	N531G
(SEQ ID NO: 24) → NG	Y20F; D425R	Δ(K1-P305); N531G;
Y408L; N531G; E457G	S250A; D425R	P535A
Y408L; N531G; E457G;	NS31G	P535G
E461Q	F537K	V311-V565-G(13)
Δ(K1-P305); Y408L;	F537R	(SEQ ID NO: 29)-
N531G; E457G; E461Q;	Q467K	K1-G310
R462K; F464Y	Q467R	C309S
Y408L; N531G; P535A	Q467K; F537K	C435S
Δ(K1-P305);	E530G	Y408L; N531G; E457G;
I317D; R318D; R319D;	E530G; N531G	E461Q; R462K
K320D; R321K	E530F	Y408L; N531G; E457G;
K14A; D425R	E530Y	E461Q; F464Y
Y408G	E530D; F537K	Y408L; N531G; E457G;
Y408P	R557K	R462K; F464Y
Y408D	P558L	Y408L; N531G; E461Q;
Y408A	E457G; E461Q	R462K; F464Y
Y408N	R462A	Y408L; E457G; E461Q;
Y408T	R462D	R462K; F464Y
Y408K	E457G; E461G	E457G; N531G; E461Q;
Y408R	N531G; E457G; E461Q	R462K; F464Y
Y408H	W381K	Y408L; E457G; E461Q;
Y408I	N404G	R462K
P535K	DS06A	N531G; E457G; E461Q;
K534Q	D506V	F464Y
E530D; N531G; G533A;	D506E	E416A
K534Q; P535K; E536N	D506G	Y408L; N531G; E457G;
R321A	I322V	E461Q; R462K; F464Y;
N402E	I322V; V323I	S507A
N422K	R327P	H370A
A502S; K504Q; F537K	I322V; V323I; R327P	P384A
A502S	V323A	P384G
K504Q	A328C; W468C	420-DGNK-424
K504E	A328C; W468C; K326Y;	(SEQ ID NO: 30) → GG
K504R	R327N	E536A
K504A	Y408L; N531G; E461Q	N85Q
K504G	D425R; Y408L; N531G;	N159Q
K504L	E457G; E461Q; R462K;	N476Q
K504P	F464Y	N518Q
K504H	Y20F; E38A; S250A;	N52Q; N85Q; N159Q
A361G	D425A	N446Q; N476Q; N518Q
T495F; Y496L; D497E;	Y20F; E38A; S250A;	B457A
S499G; I500K	D425A; Y408L; N531G;	E457D
T495F; Y496L; D497E;	E457G; E461Q; R462K;	E457F
S499G; I500K; G533A;	F464Y	E457H
K534Q; P535K; E536N;	V311-V565	E457I
F537K	K1-G310	E457K
E457Q	F559L	E457L
E457R	I560V	E457M
E457S	Y563H	E457N
E457T	P558S; F559L; I560V;	E457P
E457W	Y563H	K458A
E457Y	P558G	G469L; R456N; E457T;
E457V	L304G; P305G; K306G;	K458A
Y408E	L307G; S308G	G469L; R456N;
K14A; Y20F; D26A;	N531D	K458A
R35A; E38A	N531E	G469L; R456N; K458A;
K14A; Y20F; D26A;	NS31F	E461G

TABLE 2-continued

Exemplary CFI Variants and CFI Variant Combinations		
Variant Description	Variant Description	Variant Description
R35A; E38A; L304G; P305G; K306G; L307G; S308G Y408M Y408Q Y408S Y408W D341A Y408V E461A E461D E461F E461G E461H E461I E461L E461M E461N E461P E461S E461T E461W E461Y E461V R456A I317D-R318D-R319D- K320D-R321K; Y408L; N531G; E457G; E461Q; R462K; F464Y K312A R314A K312A; R314A P558S I345G K340G; I345G Y372G P384A P384G W381G V390G W381G; V390G W381G; P384A; V390G W381G; P384G; V390G N404G Q409G K418G D425G K418G; D425G S465G G344R G344K G344Y T346R T346K T346H K504E K504D E530R E530K T346R; K504E; E530R T346K; K504D; E530K G344R; Y408L; N531G G344K; Y408L; N531G T346R; Y408L; N531G T346K; Y408L; N531G K504D; Y408L; N531G K504E; Y408L; N531G Y408L; E530R; N531G Y408L; E530K; N531G T346R; Y408L; K504E; E530R; N531G T346K; Y408L; K504D; E530K; N531G	NS31H NS31I NS31K N531L N531M I322T N531P N531Q N531R N531S N531T N531V N531W N531Y Y403F A405S G406R Q409D A405S; G406R; Y408L; Q409D A405S; G406A; Y408L; Q409D Q409Y Q409H G406A G406A; Y408L T377G W381A W381A; P384A W381A; AP384 G469L R456N Y408L; N531G; E457G; E461Q; R462K; F464Y; S507A E457G; S507A N531G; P535A; S507A F208Y F246Y F480Y F537Y F208Y; F246Y; F480Y; F537Y H362T; V463S; R456I; D459W; S343R H362T; V463S; R456I; D459W; S343K H362T; V463S; R456F; D459W; S343R H362T; V463S; R456I; S343R H362T; R456I; D459W; S343R H362T; R456I; S343R H362T; R456I; S343K K14A; D425R; Y408L- N531G Y408L; E457G; S507A; N531G E457G; N531G E457G; Y408L Y408L; N531G; B457G; R462K Y408L; N531G; E457G; F464Y Y408L; N531G; E461Q; R462K Y408L; N531G; E461Q; F464Y Y408L; N531G; R462K; F464Y Y408L; E457G; E461Q;	G469L; R456N; K458A; E461G; F537K K504D K504F K504I K504M K504N K504S K504T K504V K504W K504Y G406D G406E G406F G406H G406I G406K G406L G406M G406N G406P G406Q G406S G406T G406V G406W G406Y G406D; Y408L G406D; N531G G406D; P535A G406D; Y408L; N531G G406D; Y408L; P535A G406D; N531G; P535A G406D; Y408L; N531G; P535A K340G N531G; E457G; R462K; F464Y N531G; E461Q; R462K; F464Y Y408L; N531G; R462K Y408L; N531G; F464Y Y408L; E457G; E461Q Y408L; E457G; R462K Y408L; E457G; F464Y Y408L; E461Q; R462K Y408L; E461Q; F464Y Y408L; R462K; F464Y N531G; E457G; R462K N531G; E457G; F464Y N531G; R462K; F464Y E457G; E461Q; R462K E457G; E461Q; F464Y E457G; R462K; F464Y E461Q; R462K; F464Y Y408L; N531G Y408L; E461Q Y408L; R462K Y408L; F464Y N531G; E461Q N531G; R462K N531G; F464Y E457G; R462K E457G; F464Y E461Q; R462K E461Q; F464Y R462K; F464Y Y408L; N422K E457G; N422K

TABLE 2-continued

Exemplary CFI Variants and CFI Variant Combinations		
Variant Description	Variant Description	Variant Description
Y408L; S507A; NS31G	F464Y	N531G; N422K
E457G; N531G; N422K	Y408L; E457G; R462K;	P535G; N422K
Y408L; E457G; N531G;	F464Y	Y408L; P535G; N422K
N422K	Y408L; E461Q; R462K;	E457G;
Y408L; E457G; P535G;	F464Y	P535G; N422K
N422K	N531G; E457G; E461Q;	N531G; P535G; N422K
E457G; N531G; P535G;	R462K	Y408L; E457G; N422K
N422K	Y408L; N531G; D425R;	Y408L; N531G; N422K
Y408L; E457G; N531G;	E530Y	K424N
P535G; N422K	E457G; N531G; D425R;	K424Q
Y408L; E416A	E530Y	K424R
E457G; E416A	Y408L; E457G; NS31G;	K424H
N531G; E416A	D425R; E530Y	K424D
P535G; E416A	D425R; Y408L; N531G;	K424E
Y408L; D425R; E416A	E457G; E461Q; R462K;	K423G
E457G; D425R; E416A	F464Y; E530Y	K423A
N531G; D425R; E416A	E457G; N531G; E461Q;	K423E
Y408L; E457G; E416A	R462K; F464Y; E530Y	K423D
Y408L; NS31G; E416A	R365A	D549A
E457G; NS31G; E416A	R365V	D549V
Y408L; E457G; N531G;	R365I	D549L
E416A	R365L	D549M
Y408L; E457G; D425R;	R365M	D549F
E416A	R365F	D549Y
Y408L; N531G; D425R;	R365Y	D549W
E416A	R365W	D549T
E457G; N531G; D425R;	R365G	D549N
E416A	R365P	D549Q
D425R; Y408L; N531G;	R365S	D549G
E457G; E461Q; R462K;	R365T	D549P
F464Y; E416A	R365N	D549R
E457G; N531G; E461Q;	R365Q	D549H
R462K; F464Y; E416A	R365H	D549K
Y408L; E530Y	R365K	Y553A
E457G; E530Y	R365D	Y553V
N531G; E530Y	R365E	Y553I
P535G; E530Y	A366G	Y553L
Y408L; D425R; E530Y	K368G	Y553S
E457G; D425R; E530Y	K368E	Y553N
N531G; D425R; E530Y	K424A	Y553Q
Y408L; E457G; E530Y	K424V	Y553R
Y408L; N531G; E530Y	K424I	Y553H
E457G; N531G; E530Y	K424L	Y553K
Y408L; E457G; N531G;	K424M	Y553E
E530Y	K424F	R557V
Y408L; E457G; D425R;	K424Y	R557I
E530Y	K424W	R557L
R557W	K424G	R557M
R557S	K424P	R557F
R557T	K424S	R557Y
R557N	K424T	Y408F; E457G; E461Q;
R557Q	Q69G	N531G
R557G	L73G	Y408F; E457G; E461Q;
R557P	L76G	R462K; F464Y; N531G
R557H	H362G	Y408F; E457G; E461Q;
R557D	H370G	R462K; N531G
R557E	F399G	Y408F; E457G; E461Q;
T377G; N531G	E401G	F464Y; N531G
T377G; E457G	A405G	E457G; E461Q; R462K;
T377G; E461Q	R456G	F464Y; N531G; R557K
T377G; E457G; E461Q	D459G	B457G; E461Q; F464Y;
T377G; E457G; E461Q;	R484G	N531G; R557K
N531G	D501G	E530F; P558S
Y408L; N531G; R557A	A502G	E530Y; P558S
N531G; P535A; R557A	V526G	E457G; E461Q; E530F;
E457G; E461Q; R557A	S527G	N531G; P558S
N531G; E457G; E461Q;	W528G	E457G; E461Q; R462K;
R557A	F537G	F464Y; E530F; N531G;
Y408L; E457G; E461Q;	P538G	P558S
R462K; N531G; R557A	V540G	Y408L; E457G; E461Q;
N531G; P535A; R557K	Y553G	R462K; E530F; N531G;
E457G; E461Q; R557K	A342G	P558S
N531G; B457G; E461Q;	R371G	B457G; E461Q; F464Y;

TABLE 2-continued

Exemplary CFI Variants and CFI Variant Combinations		
Variant Description	Variant Description	Variant Description
R557K	R327G	E530F; N531G; P558S
Y408L; E457G; E461Q;	S343G	E457G; E461Q; E530Y;
R462K; N531G; R557K	Q373G	N531G; P558S
Y408L; N531G; ΔC-term	W375G	E457G; E461Q; R462K;
(Δ558-PFISQYNNV-565	I382G	F464Y; E530Y; N531G;
(SEQ ID NO: 25))	H383G	P558S
N531G; P535A; ΔC-term	L386G	Y408L; E457G; E461Q;
(Δ558-PFISQYNNV-565	K387G	R462K; E530Y; N531G;
(SEQ ID NO: 25))	R388G	P558S
N531G; E457G; E461Q;	I389G	Y408F; E457G; E461Q;
ΔC-term (Δ558-	I391G	R462K; E530Y; N531G;
PFISQYNNV-565	E392G	P558S
(SEQ ID NO: 25)	Y393G	E457G; E461Q; F464Y;
Y408L; E457G; E461Q;	K419G	E530Y; N531G; P558S
R462K; N531G; ΔC-term	D420G	E457G; E461Q; K504H;
(Δ558-PFISQYNNV-565	N422G	N531G
(SEQ ID NO: 25))	N460G	E457G; E461Q; R462K;
ΔC-term (Δ557-	R462G	F464Y; K504H; N531G
RPFISQYNNV-565	V463G	Y408L; E457G; E461Q;
(SEQ ID NO: 28))	D425R; P558S; E457G;	R462K; K504H; N531G
Y408F; E416A; E457G;	E461Q; N531G	E457G; E461Q; F464Y;
E461Q; R462K; N531G	D425R; E530F; E457G;	K504H; N531G
E416A; E457G; E461Q;	E461Q; N531G	B416A; E457G; E461Q;
F464Y; N531G	D425K; E530F; E457G;	N531G
T377G; E457G; E461Q;	E461Q; N531G	Y408L; E416A; E457G;
R462K; F464Y; N531G	D425R; E530F; P558G;	E461Q; R462K; N531G
T377G; Y408L; E457G;	E457G; E461Q; N531G	E457G; E461Q; N422K
E461Q; R462K; N531G	K504R; E530F; P558G;	E457G; N422K; T377G
T377G; E457G; E461Q;	E457G; E461Q; N531G	E461Q; N422K; T377G
F464Y; N531G	K504R; D425R; P558G;	N531G; E457G; N422K;
T377G; E416A; K504H	E457G; E461Q; N531G	T377G
E416A; K504H	K504R; D425R; E530F;	N531G; E461Q; N422K;
T377G; K504H	E457G; E461Q; N531G	T377G
N422K; E457G; E461Q;	R557A; N531M	E457G;
N531G	R557K; N531M	E461Q; N422K;
N422K; E457G; E461Q;	R557A; N531M; Y403F;	T377G
Q467K; N531G	K504Y	T377G;
E416A; N422K; E457G;	R557A; N531D; Y403F;	N422K;
E461Q; Q467K; N531G	K504Y	E457G;
K504R; E530F; D425K;	R557A; N531M; Y403F;	E461Q; N531G
P558S	K504Y; E457G; E461Q	D425K; Y408M
K504R; E530F; D425R;	R557A; N531G; Y403F;	D425K; E530F
P558S	K504Y; E457G; E461Q	D425K; F537K
K504R; E530F; D425R;	R557A; N531D; Y403F;	D425K; K504R
P558G	K504Y; E457G; E461Q	D425K; P558S
K504R; E530F; D425K;	R557A; N531M; Y403F;	Y408M; E530F
P558G	K504Y; E457G; E461L	Y408M; K504R
K504R; E530F; D425K;	R557A; N531M; Y403F;	Y408M; P558S
P558S; E457G; E461Q;	K504Y; E457G; E461T	E530F; F537K
N531G	R557A; N531M; Y403F;	E530F; K504R
K504R; E457G; E461Q;	K504Y; E457G; E461V	E530F; P558S
N531G	R557A; N531M; Y403F;	F537K; K504R
E530F; E457G; E461Q;	K504Y; E457N; E461Q	F537K; P558S
N531G	R557A; N531M; Y403F;	K504R; P558S
D425R; E457G; E461Q;	K504Y; E457N; E461L	D425K; Y408M; F537K
N531G	R557A; N531M; Y403F;	D425K; Y408M; K504R
D425K; E457G; E461Q;	K504Y; E457N; E461T	D425K; Y408M; E530F;
N531G	R557A; N531M; Y403F;	F537K
P558S; E457G; E461Q;	K504Y; E457N; E461V	D425K; Y408M; E530F;
N531G	N531M; Y403F; K504Y;	P558S
P558G; E457G; E461Q;	E457G; E461Q	D425K; E530F; F537K;
N531G	N422K; E461Q	K504R
K504R; E530F; E457G;	T377G; N422K	Y408M; E530F; F537K;
E461Q; N531G	N531G; E457G; T377G	K504R
K504R; D425R; E457G;	N531G; E461Q; N422K	Y408M; F537K; K504R;
E461Q; N531G	N531G; E461Q; T377G	P558S
K504R; P558S; E457G;	N531G; N422K; T377G	D425K; Y408M; E530F;
E461Q; N531G	Y403F; G406A; D425K;	F537K; K504R
E530F; P558S; E457G;	E457G; E461Q; K504Y;	D425K; Y408M; E530F;
E461Q; N531G	N531G	F537K; P558S
D425K; E530F; F537K;	D425K; E457G; E461Q;	D425K; Y408M; E530F;
K504R; P558S	N531G	K504R; P558S
D425K; Y408M; E530F;	D425K; E457G; E461Q;	D425K; Y408M; F537K;

TABLE 2-continued

Exemplary CFI Variants and CFI Variant Combinations		
Variant Description	Variant Description	Variant Description
F537K; K504R; P558S	N531G; R557A	K504R; P558S
D428K; E457G; E461Q;	R557A	N402R
K504R; N531G	Δ (V565)	H554F
D425K; E457G; E461Q;	F559Y	H554K
N531G; P558S	Δ (S308)	R371E
T377G; Y408M; N422K;	F559W	A342R
E457G; E461Q; E530F;	F559H	K51R; N52A; A55P;
N531G	L307F	T59M; R61G; F64Y
T377G; N422K; D425K;	L307H	N52A
E457G; E461Q; E530F;	L307W	A55P
N531G	L307Y	T59E
E457G; E461Q; N531G;	P433A	L73F
S507A	P433G	L73D
N531G; S507A	P433F	Δ (275-CAGFASVAQE-
E457G; S507A	Δ (Y496)	284 (SEQ ID NO: 31)) >
E461Q; S507A	Δ (D497)	CEEDRQQNIPKGLARS
N422K; S507A	Δ (Y496; D497)	AQGEAEIETE
T377G; S507A	S499G	(SEQ ID NO: 32)
D425K; S507A	S499A	R484A
Y408M; S507A	S499K	R484K
P558S; S507A	S499V	R484E
E530F; S507A	Y496L	Δ (V565) > VG
F537K; S507A	S499G; I500K	Δ (V565) > VY
K504R; S507A	S499A; I500K	E416K
Y408F; S507A	T495F; Y496L; S499G;	E416R
R557A; S507A	I500K	D395A
E416A; E457G; E461Q;	K423R	D395G
R462K; F464Y; N531G	R62A	D395K
N52Q; N159Q	R62E	D395R
N476Q; N518Q	R62L	D395A; D425A
Y408F; N531M	R62H	D395A; E416A
Y408F; K504Y	E401K	D395A; E416A; D425A
G406A; Y403F	E401R	N402D
D425K	E401A	K368D
Y403F; D425K; E457G;	E401F	K1103A
N531G	N402G	E1106A
G406A; D425K; E457G;	H948N	I1134G
E461Q; N531G	L950S	I208G
Y403F; G406A; D425K;	D988N	H383K; D385N; K387S;
E457G; E461Q; N531G	L990S	R388Q; E392Q; Y393T;
Y403F; D425K; E457G;	V1108N	D395K; K419M; D420H;
E461Q; K504Y; N531G	E1110S	G421T; N422G; D425E
Q69H; L73F; G79E;		H383K; R388Q; E392Q;
T80I; L83S; N84H		Y393T; K419M
Δ (1-KVTYTS-6		K51D; T59E; R61D
(SEQ ID NO: 33)) >		Q113IN
RSPSASDLP		I1133S
(SEQ ID NO: 34)		N422A
M186E; G187L; Y188S;		T377A
Q189N; D190G; F191L;		
D193G		

B. Fusion Constructs Comprising Complement Factor I

[0064] Provided herein are formulations for fusion constructs comprising a first component (CFI portion) comprising at least one domain of complement factor I, and at least a second component, wherein the first component and second and subsequent components are fused (e.g. contiguous or separated by an optional linker). These fusion constructs are referred to herein as “CFI fusion constructs” or simply as “fusion constructs.” In some embodiments, the fusion construct comprises additional components, e.g. a third component, a fourth component, etc.

[0065] In some embodiments, the second and subsequent components of the fusion construct is a protein. In some embodiments, the second and/or subsequent components is not a protein.

[0066] The components of the fusion constructs of the disclosure may be held together by optional linkers. They may be of any suitable length of at least one amino acid. A linker may be a flexible linker, and may be a peptide of about 1 to about 20 amino acid residues in length, wherein the amino acid residues may comprise glycine residues. The linker may also optionally comprise serine residues. Exemplary flexible linkers can include, but are not limited to, glycine polymers, glycine-serine polymers, glycine-alanine polymers, alanine-serine polymers, or any other suitable flexible linkers known in the art. An exemplary linker is (GGSS)ⁿ, wherein n is any number from about 1 to about 20 (SEQ ID NO: 35). An exemplary linker is (GGSS)ⁿGG (SEQ ID NO: 36), wherein n is any number from about 1 to about 20. An exemplary linker is (GGSSG)ⁿ, wherein n is any number from about 1 to about 20 (SEQ ID NO: 37). In

some embodiments, the linkers are protease-sensitive cleavable linkers. Exemplary linkers linking the fusion constructs can be 1-50, 5-50, 10-50, 15-50, 20-50, 25-50, 1-20, 2-20, 3-20, 4-20, 5-20, 6-20, 7-20, 8-20, 9-20, 10-20, 3-15, 3-10, 3-9, 3-8, 3-7, 3-6, 3-5, 4-15, 4-10, 4-9, 4-8, 4-7, 4-6, 4-5, 5-15, 5-10, 5-9, 5-8, 5-7, 5-6, 6-15, 6-10, 6-9, 6-8, or 6-7 amino acids in length.

CFI+Half-Life Extender Fusion Constructs

[0067] In some embodiments, the fusion construct comprises a wild type CFI or CFI variant (first component), and at least a second component, and wherein the second component is a half-life extender. Because naturally occurring CFI has a relatively short half-life, it may be advantageous in some embodiments to increase the half-life of CFI or a variant thereof. By using a second component that is a half-life extender, the activity may increase, or it may improve another characteristic as compared to a wild type CFI. For example, a wild type CFI or a CFI variant may have their half-life extended by fusing the CFI to a half-life extender.

[0068] Exemplary half-life extenders include, but are not limited to albumin, such as human serum albumin, PEG, a non-biodegradable polymer, a biodegradable polymer, and Fc. In some embodiments, the second component is a protein, and is a half-life extender, such as albumin or Fc. In some embodiments, the second component is not a protein, and is a half-life extender, such as PEG. In some embodiments, the half-life extender is comprising peptide repeats.

[0069] In some embodiments, a second component is a half-life extender, and is albumin. It is noted that as used herein, albumin refers to any albumin such as any serum albumin, or an albumin variant, or albumin derivative. As an example, a variant of albumin includes any albumin comprising at least one modification corresponding to the amino acid sequence set forth in SEQ ID NO: 7 (wild type Human serum albumin (HSA)), or at least one modification corresponding to the amino acid sequence of an albumin of any non-human species. In exemplary embodiments, the albumin is human serum albumin (HSA) and is provided in SEQ ID NO: 7.

[0070] Exemplary fusion constructs comprising wild type CFI and HSA are referred to herein, as “CFI-HSA” and are discussed in further detail below. An exemplary fusion construct of the disclosure comprises the amino acid sequence of SEQ ID NO: 21.

[0071] In some embodiments, a fusion construct of the disclosure comprises albumin and a CFI variant of the disclosure. Exemplary CFI variants are provided in Table 2.

Structural Arrangements of Fusion Constructs

[0072] In some embodiments, provided herein are fusion constructs comprising at least a first component, wherein the first component is any of the wild type CFI or CFI variants provided herein (CFI portion), and a second component, wherein the first component and second component are fused, and wherein the second component is fused to the N-terminal end of the CFI portion. In some embodiments, the second component is fused to the C-terminal end of the CFI portion. In some embodiments, the second component is fused to the C-terminal end of the CFI portion, and a third component is further fused to the N-terminal end of the CFI portion. In some embodiments, the second component is fused to the N-terminal end of the CFI portion, and a third component is further fused to the C-terminal end of the CFI portion.

[0073] Turning to Table 3, SEQ ID NO: 1 is the amino acid sequence of wild type plasma-derived human CFI, referred to as “CFI-PD”, and has a leader sequence. Wild type CFI used for fusion with a second component may comprise the amino acid sequence of SEQ ID NO: 5, which does not include the leader sequence present in SEQ ID NO: 1. A mouse Ig kappa chain V-III region MOPC 63 leader sequence (SEQ ID NO: 2) may instead be used for the recombinant production of any of the CFI fusion constructs provided herein. In some embodiments, provided herein are CFI fusion constructs comprising at least one CFI domain, wherein the at least one CFI domain comprises the amino acid sequence set forth in SEQ ID NO: 5.

[0074] An exemplary fusion construct of the disclosure comprises the amino acid sequence of SEQ ID NO: 21.

TABLE 3

Components of Exemplary CFI Fusion Constructs	
Description	Sequence
Wild type plasma-derived human CFI (CFI-PD)	MKLLHVFLLFLCFHLRFCKVITYTSQEDLVEKKCLAKKYTHLSCDKVFCQP WQRCIEGTCVCKLPYQCPKNGTAVCATNRRSFPTYCQKSLACLHPGKFL NNGTCTAEGKFSVSLKHGNTDSEGIVEVKLVQDKTMFICKSSWSMREAN VACLDLGFQGGADTQRRFKLSDLINSTCLHVHCRGLETSLAECTFTKRRT MGYQDFADVVCYTQKADSPMDDFQCVNGKYISQMKACDGINCDGQSD ELCKKACQKGFHCKSGVCI PSQYQCNGEVDCITGEDEVGCAGFASVTQEE TEILTADMDAERRRIKSLPLKSCGVKNRMHIRKRIVGGKRAQLGDLPWQ VAIKDASGITCGGIYIGGCWILTAHCLRASKTHRYQIWTTVVDWIHPDLK RIVIEYVDRIIFHENYNAGTYQNDIALIEMKKDGNKKDCELPRIIPACVPWSP YLFQPNDCIVSGWGEKDNERVFSLQWGEVKLISNCSKFYGNRFYEKEM ECAGTYDGSIDACKGDSGGPLVCMDANNVTYVWGVVSWGENCGKPEFPFG VYTKVANYFDWISYHVGRPFISQYNV (SEQ ID NO: 1)
Leader sequence (mouse leader for CFI-HSA)	METDTLLLVLLLVPGSTG (SEQ ID NO: 2)
Human CFI leader sequence	MKLLHVFLLFLCFHLRF (SEQ ID NO: 3)

TABLE 3-continued

Components of Exemplary CFI Fusion Constructs	
Description	Sequence
Wild type CFI of SEQ ID NO 1 without signal sequence	KVTYTSQEDLVEKKCLAKKYTHLSCKVFCQPWQRCIEGTCVCKLPYQCP KNGTAVCATNRRSFPTYCQQKSLCLHPGKFLNNGTCTAEGKFSVSLKHG NTDSEGIVEVKLVQDKTMFICKSSWSMREANVACLDLGFQGGADTQRRF KLSDLINSSTECLHVCRLGLETSLAECTFTKRRTMGYQDFADVVCYTQKAD SPMDDFFQCVNGKYISQMKACDGINDCGDQSDCLCKACQKGFHCKSGV CIPSYQCNGEVDCTITGEDEVGCAGFASVTQEETEILTADMDAERRRIKSL PKLSCGVKNRMHIRKRIVGGKRAQLGDLFPWQVAIKDASGITCGGIYIGGC WILTAACHLRASKTHRYQIWTTVVDWIHPDLKRIVIEYVDRIIFHENYNAGT YONDIALIEMKKDGNKKDCELPKRSIPACVPWSPYLPQPNDTCTIVSGWGREK DNERVFSLOWGEVKLISNCSKFYGNRFYEKEMECAGTYDGSIDACKGDSG GPLVCMDDANNVTYVWGVVSWGENCGKPEFPGVYTKVANYFDWISYHVG RPFISQYNV (SEQ ID NO: 5)
Linker	GGSSGG (SEQ ID NO: 6)
Human serum albumin (HSA)	DAHKSEVAHRFKDLGEENFKALVLIAPAYLQCCPFEDHVKLVNEVTEFA KTCVADESAENCDSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNE CFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKLYEIAARRHPYFYA PELLFFAKRYKAAFTTECCQAADKAACLLPKLDELDEGKASSAKQRLKCA SLQKFGERAFAKAWAVARLSQRFPAEFAEVSCLVTDLTKEVTECHGDL ECADDRADLAKYICENQDSISSKLEKCEKPLEKSHCIAEVENDEMPADLP SLAADFVESKDVCKNYAEAKDVFLGMFLYFYARRHPDYSVVLRLAKTY ETTLKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCLEFQGLGEYKFQ NALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAEDYLS VVLNQLCVLHEKTPVSDRVTKCTESLVNRRPCFSALEVDETYVPKEFNAE TFTFHADICTLSEKERQIKKQTALEVLVKKPKATKEQLKAVMDDFAAFVE KCKKADDEKTCFAEEGKKLVAAASQAALGL (SEQ ID NO: 7)
HSA linked with CFI (CFI-HSA)	DAHKSEVAHRFKDLGEENFKALVLIAPAYLQCCPFEDHVKLVNEVTEFA KTCVADESAENCDSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNE CFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKLYEIAARRHPYFYA PELLFFAKRYKAAFTTECCQAADKAACLLPKLDELDEGKASSAKQRLKCA SLQKFGERAFAKAWAVARLSQRFPAEFAEVSCLVTDLTKEVTECHGDL ECADDRADLAKYICENQDSISSKLEKCEKPLEKSHCIAEVENDEMPADLP SLAADFVESKDVCKNYAEAKDVFLGMFLYFYARRHPDYSVVLRLAKTY ETTLKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCLEFQGLGEYKFQ NALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAEDYLS VVLNQLCVLHEKTPVSDRVTKCTESLVNRRPCFSALEVDETYVPKEFNAE TFTFHADICTLSEKERQIKKQTALEVLVKKPKATKEQLKAVMDDFAAFVE KCKKADDEKTCFAEEGKKLVAAASQAALGLGGSSGGKVTYTSQEDLVEKK LAKKYTHLSCKVFCQPWQRCIEGTCVCKLPYQCPKNGTAVCATNRRSFP TYCQQKSLCLHPGKFLNNGTCTAEGKFSVSLKHGNTDSEGIVEVKLVQD DKTMFICKSSWSMREANVACLDLGFQGGADTQRRFKLSDLINSSTECLHVC RGLTSLAECTFTKRRTMGYQDFADVVCYTQKADSPMDDFFQCVNGKYI SQMKACDGINDCGDQSDCLCKACQKGFHCKSGVICPSQYQCNGEVDCTI TGEDEVGCAGFASVTQEETEILTADMDAERRRIKSLPKLSCGVKNRMHIR RKRIVGGKRAQLGDLFPWQVAIKDASGITCGGIYIGGCWILTAACHLRASKT HRYQIWTTVVDWIHPDLKRIVIEYVDRIIFHENYNAGTYQNDIALIEMKKDG NKKDCELPKRSIPACVPWSPYLPQPNDTCTIVSGWGREKDNERVFSLOWGEVK LISNCSKFYGNRFYEKEMECAGTYDGSIDACKGDSGGPLVCMDDANNVTYV WGVVSWGENCGKPEFPGVYTKVANYFDWISYHVGRRPFISQYNV (SEQ ID NO: 21)

III. Uses of CFI-Containing Formulations

[0075] The formulations of the disclosure may be used for therapeutics in a subject. As used herein, a subject includes any mammalian subject and includes primates, rodents, domestic animals, zoo animals, and pets. In some embodiments, the mammalian subject is a human subject. In some embodiments, the mammalian subject is a non-human primate.

A. Treatment of Non-Ocular Conditions

[0076] In some embodiments, the formulations provided herein are useful for treating a non-ocular condition in a subject. In some embodiments, provided herein is a method

of treating an ocular condition in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of any one of the formulations provided herein.

[0077] In some embodiments, the non-ocular condition is characterized by a deficiency of CFI. In some embodiments, the non-ocular condition is characterized by dysregulation of the complement system.

[0078] In some embodiments, the non-ocular condition is a systemic acute indication. In some embodiments, the non-ocular condition is a systemic acute indication selected from the group consisting of: acute glomerulonephritis, acute renal injury, acute respiratory distress syndrome, bacterial meningitis, brain hemorrhage, burns, coronavirus

infection, Epstein-Barr virus infection, hematopoietic stem cell transplantation, ischemia reperfusion injury, Lyme disease, myocardial infarction, organ transplantation, periodontitis, pneumonia, pre-eclampsia, schistosomiasis, sepsis, stroke, thromboembolism, and traumatic brain injury.

[0079] In some embodiments, the non-ocular condition is a systemic chronic indication. In some embodiments, the non-ocular condition is a systemic chronic indication selected from the group consisting of: Alzheimer's disease, anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis, antiphospholipid syndrome, asthma, atherosclerosis, atypical hemolytic uremic syndrome (aHUS), autoimmune hemolytic anemia, bullous pemphigoid (BP), C3 glomerulopathy, chronic kidney failure, chronic obstructive pulmonary disease (COPD), Cold agglutinin disease (CAD), Crohn's disease, diabetic neuropathy, generalized myasthenia gravis (gMG), Granulomatosis with Polyangiitis (GPA), Guillain-Barré Syndrome (GBS), hereditary angioedema (HAE), hidradenitis suppurativa (HS), IgA nephropathy (IgAN), lupus nephritis (LN), membranous glomerulonephritis (MN), microscopic polyangiitis (MPA), motor neuron disease, multifocal motor neuropathy (MMN), multiple sclerosis (MS), non-insulin dependent diabetes, osteoarthritis, pancreatitis, Parkinson's disease, paroxysmal nocturnal hemoglobinuria (PNH), post-transplant lymphoproliferative disease, protein losing enteropathy, psoriasis, pyoderma gangrenosum, rheumatoid arthritis, schizophrenia (SZ), systemic lupus erythematosus (SLE), immune thrombocytopenia (ITP), and ulcerative colitis, Lampert-Eaton myasthenic syndrome (LEMS), CHAPLE syndrome (CD55 deficiency), thrombotic microangiopathy (TMA) and chronic inflammatory demyelinating polyneuropathy (CIDP), Huntington disease and ischemia reperfusion injuries.

[0080] In some embodiments, the diseases that may be treated by use of the formulations provided herein that comprise CFI variants or fusion constructs that are C4b degraders include, but are not limited to a non-ocular condition. In some embodiments, the non-ocular condition is a systemic chronic indication. In some embodiments, the non-ocular condition is a systemic chronic indication selected from the group consisting of: Alzheimer's disease, Amyotrophic lateral sclerosis (ALS), anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis, antiphospholipid syndrome, asthma, atherosclerosis, atypical hemolytic uremic syndrome (aHUS), autoimmune hemolytic anemia, bullous pemphigoid (BP), C3 glomerulopathy, chronic kidney failure, chronic obstructive pulmonary disease (COPD), Cold agglutinin disease (CAD), Crohn's disease, diabetic neuropathy, generalized myasthenia gravis (gMG), Granulomatosis with Polyangiitis (GPA), Guillain-Barré Syndrome (GBS), hereditary angioedema (HAE), hidradenitis suppurativa (HS), IgA nephropathy, lupus nephritis (LN), membranous glomerulonephritis (MN), microscopic polyangiitis (MPA), motor neuron disease, multifocal motor neuropathy (MMN), multiple sclerosis (MS), non-insulin dependent diabetes, osteoarthritis, pancreatitis, Parkinson's disease, paroxysmal nocturnal hemoglobinuria (PNH), post-transplant lymphoproliferative disease, protein losing enteropathy, psoriasis, pyoderma gangrenosum, rheumatoid arthritis, schizophrenia (SZ), systemic lupus erythematosus (SLE), immune thrombocytopenia (ITP), warm Autoimmune hemolytic anemia (wAIHA), Immune-Complex Membranoproliferative Glomerulonephritis (IC-MPGN), and ulcerative colitis, Lampert-Eaton myasthenic

syndrome (LEMS), CHAPLE syndrome (CD55 deficiency), thrombotic microangiopathy (TMA) and chronic inflammatory demyelinating polyneuropathy (CIDP), Huntington disease and ischemia reperfusion injuries.

[0081] In some embodiments, the non-ocular condition is non-oncological.

[0082] In some embodiments, the non-ocular condition is oncological. In some embodiments, the non-ocular condition is oncological, and is characterized by solid tumors, or by liquid tumors. In some embodiments, the non-ocular condition is characterized by solid tumors, and is selected from the group consisting of: colorectal tumors, hormone-refractory prostate cancer, melanoma, metastatic breast cancer, metastatic colorectal cancer, metastatic esophageal cancer, metastatic pancreas cancer, metastatic stomach cancer, nasopharyngeal carcinoma, non-small cell lung cancer, pancreas tumors, squamous cell carcinoma, and stomach tumors. In some embodiments, the non-ocular condition is characterized by liquid tumors, and is selected from the group consisting of: acute myelogenous leukemia, B-cell lymphoma, and Hodgkin's disease.

B. Treatment of Ocular Conditions

[0083] In some embodiments, the formulations provided herein are useful for treating an ocular condition in a subject. In some embodiments, provided herein is a method of treating an ocular condition in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of any of the formulations provided herein.

[0084] In some embodiments, the ocular condition is characterized by a deficiency of CFI. In some embodiments, the ocular condition is characterized by dysregulation of the complement system.

[0085] In some embodiments, the ocular condition is characterized by the presence of a dysfunctional CFI gene. In some embodiments, the ocular condition is characterized by dysregulation of the complement system and low CFI levels.

[0086] In some embodiments, the ocular condition selected from the group consisting of: diabetic macular edema (DME), diabetic retinopathy, dry age-related macular degeneration (AMD), glaucoma, keratoconjunctivitis, neuromyelitis optica spectrum disorder (NMOSD), open angle glaucoma, polypoidal choroidal vasculopathy, Stargardt Disease, uveitis, and vitreoretinopathy.

[0087] In some embodiments, wherein the ocular condition is non-oncological.

C. Administration

[0088] The in vivo administration of the formulations described herein may be carried out intravenously or subcutaneously.

[0089] In exemplary embodiments, administration of the formulations described herein is a subcutaneous administration. In some embodiments, the subcutaneous administration is a daily, every other day, twice weekly, or weekly administration.

[0090] In some embodiments, administration of the formulations described herein is an intravenous administration.

[0091] As generally contemplated herein, the CFI variants or fusion constructs described herein are delivered in an activated two chain form. However, in some instances,

Furin Activation

[0100] CFI-HSA is expressed as an inactive, single chain precursor protein, and is activated by furin, another serine protease. Furin is an endoprotease that cleaves CFI at its conserved RRKR sequence (also referred to as the furin recognition sequence), resulting in a heavy and light chain connected by a disulfide bond. The furin-processed, mature, two-chain protein is the activated form of the CFI protein.

[0101] Cleavage of CFI-HSA for producing the protein in its activated form was performed by incubation of 4 µg of recombinant furin per mg of purified CFI-HSA in Tris-NaCl (tris buffered saline), 2.5 mM CaCl₂ and 0.5% CHAPS at 30° C. for 18 hours. The CFI-HSA protein concentration was maintained at 1.4 mg/ml. This results in more than 90% activation of the protein. The activated protein was separated from inactivated CFI-HSA, and other proteins by size-exclusion chromatography. Size exclusion chromatography (SEC) was performed using a HiLoad 16/600 Superdex 200 column (GE Healthcare Life Sciences) and phosphate buffer saline (PBS, 137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 2 mM KH₂PO₄, pH 7.4) as the mobile phase. Collected fractions were analyzed by CE-SDS (LabChip GXII, Perkin Elmer). Fractions containing the target protein were pooled and analyzed by SE-UPLC.

In Vitro Sialylation

[0102] The activated CFI-HSA protein was subject to in vitro sialylation. Briefly, the sialylation was carried out in a two-step enzymatic reaction. First, a galactosylation reaction of CFI-HSA was performed in a 200 µl volume utilizing a 1:200 molar ratio of galactosyltransferase (GalT1) enzyme and CFI-HSA in 10 mM UDP-Galactose, 5 mM MnCl₂, and 100 mM MES, pH 6.5 buffer. Galactosylated CFI-HSA was purified from the reaction mixture by CaptureSelect™ Human Albumin affinity chromatography, as described earlier. Next, the sialylation reaction was performed in a 250 µl volume utilizing a 1:50 molar ratio of enzyme alpha 2,6-sialyl transferase and purified CFI-HSA in 80 µM Alkaline phosphatase, 6.1 mM CMP-NANA, 10 mM ZnCl₂ and 200 mM MES buffer, pH 6.5 at 37° C. for 1 hour. The sialylated CFI-HSA protein was purified from the reaction mixture by CaptureSelect™ Human Albumin affinity chromatography. The extent and characteristics of the sialic acid chain on CFI-HSA was determined by utilizing an Agilent/Prozyme Analytical service, GS-SAP method for total sialic acid quantitation (Agilent GS48), and mass spectrophotometric (MS) analysis (Lake Pharma analytical service), described in further detail below.

[0103] Briefly, total sialic acid quantitation was performed by mixing 20 µl of each sample with 10 µl of release reagent in a 96 well plate. The reaction mixture was incubated for 2 hours at 80° C. The samples were cooled to room temperature and 10 µl of labeling reagent was added to each sample for a further incubation of 3 hours at 50° C. The samples were again cooled down to room temperature and 160 µl of de-ionized (dI) water was added to bring the total volume to 200 µl. 10 µl of sample was injected in the Agilent UHPLC Poroshell C18 column to run at a flow rate of 0.4 ml/minute at 30° C. in 4% methanol, 8% acetonitrile in water (Line A1) and 100% ACN (Line B1). The peaks were recorded at 373/448 nm wavelength. A standard curve of total peak area versus picomoles (pmol) of sialic acid was generated by running 1-2000 pmol of NANA (N-acetylneuraminic acid,

Neu5Ac) supplied with the kit on the same column. Total sialic acid of each sample was quantitated by comparing the peak area of samples against the standard curve.

[0104] The mass spectrometric analysis was performed by a standard trypsin Q-TOF mass spectrometer. Briefly, all samples were treated, reduced and alkylated by DTT and iodoacetamide, followed by trypsin digestion. The digested samples were analyzed by Waters ACQUITY UPLC coupled to a Xevo G2-XS-QTOF mass spectrometer using a protein BEH C18 column

Polishing

[0105] Purified CFI-HSA protein was subjected to size-exclusion chromatography (SEC) using a HiLoad 16/600 Superdex 200 column (GE Healthcare Life Sciences) and phosphate buffer saline as the mobile phase. Collected fractions were analyzed by CE-SDS (LabChip GXII, Perkin Elmer). Fractions containing the target protein were pooled, and the concentration was brought to 5 mg/ml, and the samples were flash frozen for storage at -80° C.

Expression and Purification of CFI-HSA Variants

[0106] The DNA of CFI-HSA variants was generated either by synthesis or by site-directed mutagenesis utilizing standard techniques. The proteins were expressed in 250 ml of suspension in TunaCHO™ cells, as described herein with reference to wild type CFI-HSA protein, with the exception that the expression was done for 7 days instead of 14 days. After 7 days, the cells were centrifuged, and conditioned media was passed through a gravity flow column of CaptureSelect™ human albumin affinity matrix (ThermoFisher Scientific). Column-bound protein was washed with 10 column volume of 20 mM sodium phosphate buffer. Bound CFI-HSA protein was eluted with 3 column volume of 20 mM Tris-HCl, pH 7.0 buffer with and 2 M MgCl₂ in 5 ml fractions. CFI-HSA or its variants were buffer exchanged (either by dialysis or a spin concentrator) into 30 mM HEPES, 150 mM NaCl, 2.5 mM CaCl₂, pH 7.4. Recombinant human furin, at a molar ratio of 1:25 (furin: CFI-HSA), was added to CFI-HSA and the reaction mixture was incubated at 30° C. for 16 hours. Two micrograms of the activation mixture was run on a 9% SDS-PAGE gel to assess the activation efficiency. Generally, more than 80% activation was achieved.

Example 2: Formulation Development Study I

[0107] For Example 2, reference to CFI-HSA refers to human serum albumin fused to the N-terminal end of wild type CFI (SEQ ID NO: 21). CFI-HSA was formulated at a concentration of about 150 mg/mL.

[0108] The study was designed to investigate the physical and chemical properties of CFI-HSA, in a range of liquid and lyophilized formulations to assess the conditions that provide optimal options for stability. For example, to enable subcutaneous administration, a high concentration and stability of a CFI containing composition would be desired.

[0109] The active pharmaceutical ingredient (API) examined in this study was CFI-HAS (SEQ ID NO: 21). The material used for this study included the following:

[0110] (1) Drug Substance: CFI-HSA, 153.4 mg/mL, F1 formulation

[0111] (2) The chemicals and materials used to formulate and analyze CFI-HSA were as follows, show in Table 2.1:

[0119] Formulations were sterile-filtered through a 0.2 μ m PES membrane in an aseptic BSC and filled to 0.2 mL into 2 cc sterilized glass vials. Vials were then partially stoppered

TABLE 2.1

Materials				
Chemicals	Vendor	Catalog #	CAS #	Grade
Acetic acid	EMD	AX0073-9	64-19-7	
Sorbitol	EMD	111597	50-70-4	EP, BP, NF, JP
NaCl	EMD	106404	7647-14-5	EP
Succinic acid	Sigma Aldrich	1623411	110-15-6	USP
Arginine.HCl	Sigma Aldrich	A4599	1119-34-2	EP, JP, USP
Sucrose	Pfanstiehl	S-124-2-MC	57-50-1	NF, EP, JP, ChP
Trehalose	Pfanstiehl	T-104-4	6138-23-4	NF, EP, JP, ChP
Glycine	JT Baker	0581-05	56-40-6	USP
Polysorbate 80	Croda	Super Refined TM	9005-65-6	USP, JP, EP

[0112] In this stability study, the stability of CFI-HSA was monitored in twelve (12) formulations containing various tonicity modifiers/bulking agents across an optimal range of pH. Table 2.2 provides the formulation matrix for the twelve tested.

[0113] (1) pH 5-7

[0114] (2) Buffers (also referred to interchangeably herein as buffering agents)

[0115] (3) Tonicity modifiers (sorbitol or trehalose for non-ionic buffering agents, and sodium chloride for ionic buffering agents)

[0116] (4) Bulking agents (trehalose or glycine)

[0117] (5) Surfactants

with lyo vent stoppers and were lyophilized using the parameters outlined in Table 2. Following lyophilization, the chamber was backfilled with nitrogen and vials were stoppered. Vials were then removed from and stoppered, crimped, and labeled. Additional vials were set aside and frozen prior to lyophilization for pre-lyophilization analysis alongside time zero.

Liquid Formulation Preparations

[0120] For each formulation, CFI-HSA DS solution was loaded into a dialysis cassette (Slide-A-Lyzer® Dialysis

TABLE 2.2

Formulation Matrix						
Form No.	Form. Type	Buffer (20 mM)	Tonicity Modifier/ pH Bulking Agent	API (mg/m L)	Surfactant	
1	Liquid	Sodium acetate	5 5% Sorbitol	150	0.02% PS 80	
2	Liquid	Sodium acetate	5.5 5% Sorbitol	150	0.02% PS 80	
3	Liquid	Sodium acetate	5.5 150 mM NaCl	150	0.02% PS 80	
4	Liquid	Sodium acetate	5.5 70 mM arginine. HCl, 2% sucrose, 60 mM glycine	150	0.02% PS 80	
5	Liquid	Histidine HCl	6 70 mM arginine.HCl, 2% sucrose, 60 mM glycine	150	0.02% PS 80	
6	Liquid	Histidine HCl	6 150 mM NaCl	150	0.02% PS 80	
	Lyo	Sodium succinate	5 10% Trehalose	150	0.02% PS 80	
8	Lyo	Sodium succinate	5% Trehalose	150	0.02% PS 80	
			2.0% Glycine			
9	Lyo	Histidine HCl	6 10% Trehalose	150	0.02% PS 80	
10	Lyo	Histidine HCl	6 5% Trehalose, 2% Glycine	150	0.02% PS 80	
11	Lyo	Histidine HCl	10% Trehalose	150	0.02% PS 80	
12	Lyo	Histidine HCl	7 5% Trehalose, 2% Glycine	150	0.02% PS 80	

*Form. = Formulation; API = active pharmaceutical ingredient, CFI-HSA; Lyo = lyophilized

Lyophilized Formulation Preparations

[0118] For each formulation, CFI-HSA DS solution was loaded into a dialysis cassette (Slide-A-Lyzer® Dialysis Cassette, 10,000 MWCO). Formulations were dialyzed into their respective formulation buffers.

Cassette, 10,000 MWCO). Formulations were dialyzed into their respective formulation buffers. Formulations were sterile-filtered through a 0.2 μ m PES membrane in an aseptic BSC and filled to 0.2 mL into 2 cc sterilized glass vials. Vials were then stoppered, crimped, and labeled.

Analytical Methods

[0121] The following analytical methods were employed.

[0122] (1) Visual Inspection: Visual inspection was performed under a white light source (13 W fluorescent tube) against black or white background. Digital photographs were acquired of all formulations at every time point.

[0123] (2) Concentration Measurement: The concentration of CFI-HSA was analyzed by A280 via SoloVPE using an E.C. of $1.041 \text{ mg/mL}^{-1} \times \text{cm}^{-1}$.

[0124] (3) SE-HPLC: Size-exclusion HPLC chromatography was executed with the following parameters.

[0125] HPLC: Agilent 1260 system

[0126] Column: Agilent AdvanceBio SEC 300 Å, 4.6×300 mm, 2.7 µm, LC columnP/N: PL1580-5301

[0127] Mobile Phase: 150 mM Sodium Phosphate, pH 6.9±0.1

[0128] Diluent: DI water

[0129] Sample Concentration: 0.5 mg/mL

[0130] Flow Rate: 0.35 mL/min

[0131] Run Time: 15 min

[0132] Autosampler Temp.: 5° C.

[0133] Column Temp.: 30° C.

[0134] Absorbance Detection: 280 nm, 215 nm

[0135] Injection Volume: 20 µL

[0136] Injection load: 15 minutes

[0137] (4) FlowCAM: The FlowCAM particle imaging system combines optics, electronics, and fluidics for automated analysis of particles. The optical system is used to capture real-time images of the particles in the fluid as they pass through the flow cell. The imaging software provides the ability to assess particle size and morphology. All samples were analyzed and degassed for 30 minutes at 75 torr prior to analysis.

[0138] (5) Sub-Ambient DSC: Using a Pyris Diamond DSC with an Intercooler II, approximately 10 µL of each sample was frozen at -60° C. At a ramp rate of 10° C. min, the sample was warmed until thawed and the heat flow during the warming process was recorded.

versus wavenumber [cm^{-1}]. This data yields information on secondary structure of polypeptides.

Surfactant Screening

[0140] The CFI-HSA drug substance was diluted to 15 mg/mL with water. The solution was then sterile-filtered through a 0.2 µm syringe filter and filled into 3 cc vials at a fill volume of 1.0 mL. Four vials each were then directly spiked with the stock solutions of either 1% PS20, 1% PS80, or 10% F-68 to reach final concentrations of 0.015% PS20, 0.015% PS80, or 0.15% F-68. Four of the filled vials were used as a surfactant-free control. A total of four vials per condition were prepared. The vials per condition were placed on a shaker and were agitated at 1000 rpm for four hours at ambient temperature. One (1) vial per condition was concurrently incubated at ambient temperature for four hours.

TABLE 2.3

Surfactant Screen Matrix	
Surfactant	CFI-HSA (mg/mL)
None (No Surfactant)	15.3
0.015% PS20	
0.015% PS80	
0.15% F-68	

[0141] The stability of CFI-HSA with and without surfactants was studied following agitation for four hours (1000 RPM on an orbital shaker, room temperature); and static conditions at four hours room temperature).

[0142] Following agitation and static conditions, all samples appeared clear, colorless, and free of visible particulates, regardless of surfactant.

[0143] After four hours of agitation or static incubation, similar chromatographic profiles were observed for all samples with or without PS80, PS20, and F68. All samples also showed similar purity and total peak area (Table 2.4).

TABLE 2.4

Surfactant Screening SE-HPLC Results						
Sample	Static Samples		Agitated Sample 1		Agitated Sample 2	
	Percent Total Area (%)		Percent Total Area (%)		Percent Total Area (%)	
	HMW 1	Main Peak	HMW 1	Main Peak	HMW 1	Main Peak
No surfactant	0.2	99.8	0.6	99.4	0.5	99.5
Polysorbate 80	0.2	99.8	0.5	99.5	0.5	99.5
Polysorbate 20	0.2	99.8	0.6	99.4	0.4	99.6
Poloxamer F68	0.2	99.8	0.3	99.7	0.2	99.8

[0139] (6) Fourier Transform Infrared Spectroscopy (FTIR): FTIR is a technique used to obtain an infrared spectrum of absorption of a gas, liquid, or solid. IBI uses an FTIR-660 Plus spectrometer which collects absorption of infrared radiation by the sample material

[0144] FlowCam Analysis is show in Table 2.5. Samples without a surfactant shows significant increase in the particle counts. However, all surfactant-containing samples displayed relatively lower particle concentrations, regardless of surfactant.

TABLE 2.5

Surfactant Screening FlowCam Results																
Surfactants																
No surfactant				PS80 (0.02%)				PS20 (0.01%)				Pluronic F68 (0.1%)				
Agitation				Agitation				Agitation				Agitation				
Particles/mL	Static	1	2	3	Static	1	2	3	Static	1	2	3	Static	1	2	3
>2 μm	498	5111	6896	1516	648	409	1904	818	259	1504	3442	1335	80	2143	988	2232
>5 μm	90	1365	2173	608	279	109	509	120	110	578	978	309	30	668	265	578
>10 μm	50	259	239	150	10	35	70	20	20	90	190	70	10	90	49	60
>25 μm	0	10	10	0	0	7	10	0	0	10	50	0	0	20	25	0

[0145] Based on these results, it was determined that the 0.02% polysorbate 80, was one optimal choice for the next round of formulation optimization study.

Formulation Optimization

[0146] The objective of the accelerated stability study was to assess the stability of both liquid and lyophilized formulations, containing CFI-HSA at 150 mg/mL. All formulations were filled at 0.2 mL fill volumes and F7-F12 were lyophilized. Pre-lyophilized samples were frozen at -70°C . for the duration of the lyophilization cycle and were thawed at time zero for analysis.

[0147] This study included the incubation of all formulation candidates at refrigerated (5°C .), stressed (25°C .), and accelerated (40°C .) storage temperatures. The temperature storage portion of the study was performed over a four-week period. Table 2.6 summarizes storage conditions that were used for formulation stability evaluation.

TABLE 2.6

Summary of Stability Study Stress Conditions		
Stress	Conditions	Time Point(s)
Temperature (Liquid)	5°C .; 25°C .; 40°C .	0, 1, 2, 4 weeks

TABLE 2.6-continued

Summary of Stability Study Stress Conditions		
Stress	Conditions	Time Point(s)
Temperature (Lyophilized)	5°C .; 25°C .; 40°C .	
Agitation	Vortex	4 hours at 1000 RPM

[0148] At time zero, vials were placed at appropriate temperatures following Table 2.6. Vials were pulled at each time point and analyzed.

[0149] At time zero, all pre-lyophilized formulations appeared clear, colorless, and free of visible particulates. Lyophilized formulations displayed good cakes. All liquid and reconstituted lyophilized formulations appeared clear, slightly yellowish tint, and free of visible particulates. Throughout the study up to the four-week time point, no significant changes were observed in the lyophilized cakes, regardless of storage temperature. Similarly, most liquid and reconstituted lyophilized formulations appeared clear and yellow tint, with no visible particulates at all temperatures.

[0150] At time zero, all formulations displayed concentrations near the target, ranging from 130 mg/mL to 160 mg/mL. Throughout the study up to the four-week time point, most formulations at 5°C ., 25°C ., and 40°C . displayed concentrations within experimental variability. Table 2.7 shows the stability concentration results.

TABLE 2.7

Stability Concentration Results									
Concentration (mg/mL)									
Form		T = 1 week			T = 2 week			T = 4 week	
No.		T = 0	25°C .	40°C .	5°C .	25°C .	40°C .	5°C .	25°C .
1	LIQUID	150.49	150.25	148.81	144.89	145.86	147.50	148.1	143.0
2		144.67	157.14	156.21	153.71	151.85	154.11	155.5	155.8
3		143.57	149.07	145.49	117.26	116.40	109.87	117.7	118.0
4		141.64	147.39	139.79	136.98	136.45	130.40	138.3	139.5
5		147.23	151.12	147.23	130.47	133.93	125.85	130.3	132.3
6		128.91	135.09	127.99	118.92	120.16	107.19	119.3	119.4
7	LYOPH	156.46	180.31	175.71	170.60	174.47	174.83	168.9	174.2
8		158.47	160.32	150.62	142.27	151.29	152.59	153.6	153.1
9		178.19	170.42	174.25	171.15	155.01	171.56	151.3	174.2
10		143.10	141.45	146.56	147.61	146.88	147.56	145.5	146.9
11		147.09	154.29	139.70	146.20	143.73	143.09	146.8	143.6
12		128.43	127.70	126.12	128.12	127.46	127.72	127.6	127.9
13		137.87	N/A	136.39	N/A	N/A	199.96	N/A	148.8

[0151] FTIR spectrum of CFI-HSA appeared similar to what was reported from albumin with strong α -helical signal at 1650-1660 cm⁻¹. No major change in the FTIR spectrum was observed when CFI-HSA was lyophilized in the candidate formulations.

[0152] At time zero, all formulations including reconstituted lyophilized formulations displayed comparable chromatographic profiles with minor HMW (high molecular weight) and LMW (low molecular weight) peaks. (SE-HPLC results)

[0153] After 1 week storage at 25-40° C., all liquid formulations displayed increase in HMWS as well as LMW. The increase of LMW was much faster at lower pHs. All lyophilized formulations, however, remained much more stable, especially with no sign of increase of LMW. (SE-HPLC results)

[0154] After 2 week storage at 5, 25 and 40° C., all liquid formulations displayed increase in HMWS as well as LMW. The increase of LMW was much faster at lower pHs. All lyophilized formulations, however, remained much more stable, especially with no sign of increase of LMW. (SE-HPLC results)

[0155] General degradation process remained consistent at 4 week time point at 5, 25 and 40° C.: all liquid formulations displayed increase in HMWS as well as LMW. The increase of LMW was much faster at lower pHs. All lyophilized formulations, however, remained more stable, especially with no sign of increase of LMW. (SE-HPLC results)

[0156] The rates of degradation in each tested formulation over 4 week time storage at 40° C. were compared. Among all tested formulations, in this study, lyophilized formulations containing both trehalose and glycine as bulking agents performed best at pH range of 6-7.

Findings

[0157] The stability of CFI-HSA at 150 mg/mL in various formulation conditions was examined in this example. The conditions investigated included liquid and lyophilized formulations containing various buffers (20 mM sodium acetate, sodium succinate, or histidine-HCl), tonicity modifiers (sodium chloride, sorbitol, or trehalose), bulking agents (trehalose or glycine), and across pH values ranging from 5.0 to 7.0. Formulations were examined under static storage conditions at refrigerated (5° C.), ambient (25° C.), and accelerated (40° C.) temperatures for up to four (4) weeks. Over the course of the study, analysis by visual inspection, concentration, SE-HPLC, and FlowCAM was carried out. Additionally, lyophilized formulations were also analyzed by DSC and FTIR.

[0158] Initially, a small surfactant screen was performed using commonly used surfactant stabilizers. The study evaluated the benefit of surfactants in stabilization of CFI-HSA to degradation following exposure to agitation induced shear stresses. CFI-HSA drug substance at 15 mg/mL was spiked with surfactants 0.015% PS20, 0.015% PS80, and 0.15% F68. Comparison of surfactant-containing vials following the agitation stress by SE-HPLC showed comparable profiles for all samples regardless of agitation. However, MFI analysis demonstrated surfactant-free samples displayed substantially increased sub-visible particulate concentrations compared to those containing surfactant. This provided evidence that CFI-HSA was sensitive to shear stress and improved stability could be achieved with addi-

tion of surfactant in further studies. The current surfactant in the drug substance, 0.02% PS80, was selected for the accelerated stability study.

[0159] Following the preparation of the 12 candidate formulations, all samples appeared clear and free of visible particulates at time zero.

[1610] Throughout the entire study, no significant changes in visual appearance were observed in the lyophilized cakes—regardless of storage temperature. Similarly, most liquid and reconstituted lyophilized formulations appeared clear with signature yellow tint at all temperatures. Following four weeks, most formulations were near target concentration at all storage conditions.

[0161] FTIR results found no significant differences in spectra profiles between pre-lyophilized and lyophilized samples.

[0162] FlowCAM analysis saw relatively low subtracted subvisible particle concentrations for all samples, suggesting that precipitation or particle is not a critical factor for properly formulated CFI-HSA.

[0163] The accelerated study was under conditions of extreme pressure and may not be predictive. Nonetheless, analysis by SE-HPLC following four weeks at all temperatures saw significantly higher LMW in liquid formulations, especially low pH formulations like F1, F2, F3, and F4. Even at pH 6, F5 and F6 saw approximately 1% increase in LMW during storage for four weeks at 5° C. Additives that are effective in inhibiting the autolysis of CFI-HSA may be useful to include. The autolysis is stopped when CFI-HSA is lyophilized. No significant increase in the LMW was seen when the lyophilized formulations were stored for four weeks at 40° C. Among tested, formulations F8, F10, and F12 containing glycine as a bulking agent, an ionic bulking agent as compared to non-ionic trehalose, showed improved stability against the aggregation. In addition, the aggregation was slower as the pH increased from 5 to 7.

[0164] The key degradation products and stability indicating assays were identified as increases in HMW and LMW by SE-HPLC. Based on all the results generated in this study, one leading formulation for CFI-HSA at 150 mg/mL may be the lyophilized condition of 20 mM histidine, 5.0% trehalose, 2.0% glycine, and 0.02% polysorbate 80 at pH 6.0-7.0.

Example 3: Formulation Development Study II

[0165] Additional formulations for the active ingredients described herein were developed in an indepenf study. To enable subcutaneous administration, high concentration and stability of a CFI containing composition is desirable to achieve.

[0166] For Example 3, reference to CFI-HSA refers to human serum albumin fused to the N-terminal end of wild type CFI (SEQ ID NO: 21). CFI-HSA was formulated at a concentration between 30-189 mg/mL.

Biophysical Characterization and pH Stability Profile

[0167] A stock solution of CFI-HSA at 5 mg/mL was dialyzed to citrate phosphate buffer containing 135 mM sodium chloride, at pH 5, 5.5, 6, 6.5, 7, or 7.5. Following visual appearance to assess any potential precipitation, the samples were 0.2 micron filtered, adjusted to 2 mg/ml in the corresponding buffer and aseptically transferred to microtubes. Sets of samples were stored at either -70°C. 25°C.

or 40° C. After 7 days, sets were analyzed by appearance (opalescence and particles), protein concentration (OD280), SEC, DSF, DLS, icIEF, CE-SDS, and S2288 chromogenic activity assay.

[0168] Following 7 days of storage at either 25° C. or 40° C., all samples were found to be visually clear with no visibly detectable particles at both temperatures and all pH levels tested. Protein concentration was stable in all samples (Table 3.1).

TABLE 3.1

CFI-HSA concentration after one week storage at 25° C. and 40° C.				
Sample Code	pH	Temperature	Concentration (mg/mL) just after dialysis	Concentration (mg/mL) post-dialysis
			adjusted from 5 to 2 mg/ml	at one week
Sample A	5.0	25° C.	2.0	1.95
Sample A	5.0	40° C.	2.0	1.94
Sample B	5.5	25° C.	2.0	2.0
Sample B	5.5	40° C.	2.0	2.02
Sample C	6.0	25° C.	2.0	1.99
Sample C	6.0	40° C.	2.0	2.02
Sample D	6.5	25° C.	2.0	1.98
Sample D	6.5	40° C.	2.0	1.99
Sample E	7.0	25° C.	2.0	1.97
Sample E	7.0	40° C.	2.0	1.99
Sample F	7.5	25° C.	2.0	2.02
Sample F	7.5	40° C.	2.0	2.04

[0169] SEC-HPLC was used to assess aggregation under storage conditions. At 25° C. storage, the main peak purity showed a steady increase as the pH decreased from 7.5 to 5.0. On the contrary, at pH 5.0 and 40° C. storage conditions, the main peak purity had a sharp decrease and the lower molecular weight species had a spike, indicating potential protein degradation had occurred. In summary, under these conditions, a pH of between 5.5 and 6.0 appeared to be the optimal range for CFI-HSA stability.

[0170] The hydrodynamic size of the ensemble collection of particles was measured by dynamic light scattering (DLS) to further assess aggregation under storage conditions. The results showed that both the intensity weighted mean (Zave) and polydispersity increased steeply at pH 5, but were comparable between pH 6-7.5. Meanwhile, size of the major species (>99.8%) increased slightly with increasing pH. Taken together, the polydispersity and particle size data measured by DLS indicated an optimal storage pH of about 6, corroborating the SEC-HPLC results.

[0171] CE-SDS was performed to assess the purity changes using the pre-formulations. Results showed a small decrease in purity at pH 5 and 7.5 and comparable purity between pH 5.5-6.5 by both reducing and non-reducing CE-SDS. Similarly, optimum pH levels between 5.5 and 7.0 were suggested by differential scanning fluorimetry (DSF), while pH 5 was found to be the least stable by imaged capillary isoelectric focusing (icIEF).

High Concentration Liquid Formulations

[0172] Feasibility of a high concentration formulation was assessed. Purified CFI-HSA was dialyzed to 20 mM histidine, 150 mM ArgHCl, pH of 5.8, and concentrated to 189 mg/mL in a spin filter unit. A high concentration of arginine was used to increase solubility and prevent excessive viscosity caused by the concentrated CFI-HSA. To evaluate the impact of protein concentration and arginine concentration on viscosity, concentrated sample at 189 mg/mL was also diluted with 20 mM His, pH of 5.8 to 169 mg/mL to reach 135 mM ArgHCl. The data presented in Table 3.2 indicated that the viscosity was only 10.6 centipoise (CP) even at 189 mg/mL CFI-HSA, indicating minimal risk of excessive viscosity using the tested formulation.

TABLE 3.2

Viscosity of high-concentration CFI-HSA		
CFI-HSA	Buffer	Viscosity
189 mg/mL	20 mM His, 150 mM Arg.HCl, pH of 5.8	10.6 CP
169 mg/mL	20 mM His, 135 mM Arg.HCl, pH of 5.8	8.1 CP

[0173] The concentrated samples were 0.2 micron filtered and tested for aggregates using SEC. No change in aggregate level (1.5%) was observed in the 188.7 mg/mL sample in 150 mM arginine diluted to 5 mg/mL and stored in the 5° C. autosampler. The 169.7 mg/ml sample that was diluted to 5 mg/ml and stored in the 5° C. autosampler for 1 week showed only 1.72% aggregates. The 169.7 mg/mL sample after 1 week at 5° C. then diluted to 5 mg/ml showed 2.5% HMW species.

[0174] Bioactivity of the CFI-HSA sample concentrated to 169.7 mg/mL was assessed using an S2288 chromogenic assay. As shown in FIG. 20, the activity of the 169.7 mg/mL sample is comparable to that of the control (Ctl) sample. The minor difference in the slope of the curves could be due to either inaccuracy of the final concentration in the assay after significant dilutions of a viscous solution, or due to some loss in bioactivity after about 1 month storage at 5° C.

[0175] Taken together, these results suggest a liquid formulation containing arginine hydrochloride and histidine buffer can stabilize CFI-HSA at high concentration (e.g. greater than 150 mg/mL).

Formulation Screening

[0176] A panel of formulations (F1 to F7) were screened to determine the effect of ionic strength, buffer type, cryoprotectant and calcium chloride (CaCl₂) on the stability of CFI-HSA. Formulations included pharmaceutically acceptable excipients, including a range of concentrations of tonicity modifiers (also referred to interchangeably herein as tonicity modifiers), cryoprotectants, lyoprotectants, stabilizer and surfactant were added to the previously tested histidine and arginine buffer. Table 3.3 shows the formulations tested in this example, F1-F7, not to be confused with the F1-F12 of Example 2.

TABLE 3.3

Tested formulations for CFI-HSA							
Formulation	pH	Histidine Buffer (mM)	Tonicifier Arginine Hydrochloride (mM)	Cryoprotectant/ Lyoprotectant	Stabilizer	Surfactant (PS20)	Osmolarity
F1	5.8	20	135	—	—	0.02%	279
F2	5.8	20	70	4% glucose	—	0.02%	279
F3	5.8	20	—	8.5% sucrose	—	0.02%	285
F4	5.8	20	70	120 mM glycine	—	0.02%	282
F5	5.8	20	70	2.5% sorbitol	—	0.02%	299
F6	5.8	20	70	4% trehalose	—	0.02%	279
F7	5.8	20	—	5% sucrose	35 mM CaCl ₂	0.02%	279

[0177] The stock CFI-HSA sample was concentrated to about 40 mg/mL, then dialyzed against the 7 formulations listed in Table 3.3. The concentrated samples were 0.2 micron filtered and aseptically filled in glass vials and capped. One vial of each composition was subjected to 3 cycles of freezing and thawing, and assessed by appearance, OD280 and SEC. For a short term thermal stability study, four vials of each composition were included in a 5 week stability study, one each at -70°C ., 5°C ., 25°C . and 40°C . 5-week stability samples were analyzed by OD280, SEC and CE-SDS. In addition, to assess deamidation and oxidation, peptide map MS/MS and bioactivity analysis were conducted for selected samples.

[0178] After 5 weeks, visual inspection showed that all the formulations were colloiddally stable, with the exception of F3 when stored at 40°C . sample which had signs of the precipitation. Protein concentration measurements at OD280 showed the CFI-HSA concentrations remained unchanged after either three freeze/thaw cycles 1-week, or 5-week storage at all tested temperatures.

[0179] Assessment of purity by SEC and CD-SDS showed that for all 7 formulations, -70°C . samples were unchanged in purity, 5°C . samples have about 97% purity, 25°C . samples have about 96%, and all 40°C . samples are between 90-92% purity of the main species. The significant decrease in purity at 40°C . and slight decrease in purity at 25°C . over 5 weeks of storage in all 7 formulations indicate that a liquid high concentration formulation at room temperature is not feasible.

[0180] Bioactivity assays were performed to verify that CFI-HSA maintained activity following storage in the tested formulations and storage conditions (Table 3.4). The formulations are overall relatively comparable. Formulation F1 is

slightly more stable than the other six formulations at 40°C . Formulation F3 is the least stable at both 25°C . and 40°C ., which showed 20-30% lower activity compared to -70°C . controls, and a greater loss of activity than F2, F4, or F1 samples.

TABLE 3.4

Bioactivity of CFI-HSA following tested storage conditions							
	F1	F2	F3	F4	F5	F6	F7
5°C .	113%	94%	92%	99%	107%	112%	107%
25°C .	116%	83%	72%	89%	85%	90%	—
40°C .	62%	35%	26%	32%	35%	38%	—

[0181] Peptide mapping MS/MS was performed for the CFI-HSA formulations to determine the level of deamidation and oxidation following the tested storage conditions. Formulations F1, F2, F4, F5, and F6 following storage for 5 weeks at 5°C . samples were tested. An F4 (F/T) frozen was run as a control. The results indicated that deamidation and oxidation levels on all sites are comparable among different formulations.

[0182] Additional testing and formulations developed for clinical evaluation and drug manufacturing include formulations in Table 3.5. CFI-HSA was buffer exchanged to the 3 new formulations in Table 3.5 and concentrated to 100 mg/mL or 150 mg/mL while observing any precipitation, severe opalescence, or high viscosity. To make the formulations more suitable for lyophilized product, 60 mM glycine or 60 mM mannitol were added to F2 and F3 to protect the structure of the lyophilized cake, respectively. Samples were then sterile filtered and file, and tested for viscosity, SEC and activity.

TABLE 3.5

Formulations for clinical manufacturing and testing							
	Prot. Conc. (mg/mL)	pH	Buffer	Tonicifier/Cryo/Lyoprotectant			PS-80
F1	100	5.8	20 mM	135 mM Arg HCL			0.02%
F2			Histidine	70 mM Arg HCL	2% sucrose	60 mM Glycine	0.02%
F3				70 mM Arg HCL	2% sucrose	60 mM Mannitol	0.02%
F1	150			135 mM Arg HCL			0.02%
F2				70 mM Arg HCL	2% sucrose	60 mM Glycine	0.02%
F3				70 mM Arg HCL	2% sucrose	60 mM Mannitol	0.02%

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CLHVHCRGLE	TSLAECTFTK	240
CDGINDCGDQ	SDELCCACKQ	300
QEETEILTAD	MDAERRRIKS	360
SGITCGGIYI	GGCWILTAAH	420
YNAGTYQNDI	ALIEMKKDG	480
VFSLQWGEVK	LISNCSKFY	540
WGVVSWGENC	GKPEFPGVYT	583
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CQKGKGFHCK	S	300
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AHCLRASKTH	RYQIWTTVVD	420
GNKKDCELPR	SIPACVPWSP	480
YGNRFYEKEM	ECAGTYDGS	540
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	THLSCDKVFC	
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CQGGKFHCKS	GVCIPSQYQC	NGEVDCTIGE	DEVGCAFGAS	VTQEETEILT	ADMDAERRRI	300
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EGKFSVSLKH	GNTDSEGIVE	VKLVDQDKTM	FICKSSWSMR	EANVACLDLG	FQQGADTQRR	720
FKLSDLINS	TECLHVHCRG	LETSLAECTF	TKRRTMGYQD	FADVVCYTQK	ADSPMDDFFQ	780
CVNGKYISQM	KACDGINDCG	DQSDDELCCA	CQGGKFHCKS	GVCIPSQYQC	NGEVDCTIGE	840
DEVGCAFGAS	VTQEETEILT	ADMDAERRRI	KSLLPKLSG	VKNRMHIRRK	RIVGGKRAQL	900
GDLPWQVAIK	DASGITCGGI	YIGGCWILTA	AHCLRSKTH	RYQIWTTVVD	WIHPDLKRIV	960
IEYVDRIIFH	ENYNAGTYQN	DIALIEMKKD	GNKKDCELPR	SIPACVPWSP	YLFQPNDTCI	1020
VSGWGREKDN	ERVFSLOWGE	VKLISNCSKF	YGNRFYEKEM	ECAGTYDGS	DACKGDSGGP	1080
LVCMDANNVT	YVWGVVSWGE	NCGKPEFPGV	YTKVANYFDW	ISYHVGRPFI	SQYNV	1135
SEQ ID NO: 17	moltype =		length =			
SEQUENCE: 17						
000						
SEQ ID NO: 18	moltype =		length =			

-continued

SEQUENCE: 18		
000		
SEQ ID NO: 19	moltype =	length =
SEQUENCE: 19		
000		
SEQ ID NO: 20	moltype =	length =
SEQUENCE: 20		
000		
SEQ ID NO: 21	moltype = AA	length = 1156
FEATURE	Location/Qualifiers	
source	1..1156	
	mol_type =	protein
	organism =	synthetic construct
SEQUENCE: 21		
DAHKSEVAHR	FKDLGEENFK	ALVLIIFAQY LQQCPFEDHV KLVNEVTEFA KTCVADESAE 60
NCDKSLHTLF	GDKLCTVATL	RETYGEMADC CAKQEPERNE CFLQHKDDNP NLPRLVRPEV 120
DVMCTAFHDN	EETFLKKYLY	EIARRHPYFY APELLFFAKR YKAAFTECCQ AADKAACLLP 180
KLDELRDGK	ASSAKQRLKC	ASLQKFGERA FKAWAVARLS QRFPKAEFAE VSKLVTDLTK 240
VHTECCHGDL	LECADDRADL	AKYICENQDS ISSKLKECCE KPLLEKSHCI AEVENDEMPA 300
DLPSLAADFV	ESKDVCKNYA	EAKDVFLGMF LYEYARRHPD YSVVLLRLA KTYETTLEKC 360
CAAADPHECY	AKVFDEFKPL	VEEPQNLIQ NCELFEQLGE YKFQNALVR YTKKVPQVST 420
PTLVEVSRNL	GKVGSKCKH	PEAKRMPCAE DYLSVVLNQL CVLHEKTPVS DRVTKCCTES 480
LVNRRPCFSA	LEVDETYVPK	EFNAETFTPH ADICTLSEKE RQIKKQTALV ELVKHKPKAT 540
KEQLKAVMDD	FAAFVEKCKK	ADDKETCFAE EGKKLVAASQ AALGLGGSSG GKVTYTSQED 600
LVEKKCLAKK	YTHLSCDKVF	CQPWQRCIEG TCVCKLPYQC PKNGTAVCAT NRRSFPTYCQ 660
QKSLECLHPG	TKFLNNGTCT	AEGKFSVSLK HGNTDSEGI VEVKLVDQDKT MFICKSSWSM 720
REANVACLDL	GFQGGADTQR	RFKLSDLISN STECLHVHCR GLETSLAECT FTKRRTMGYQ 780
DFADVVCYTQ	KADSPMDDF	CQVNGKYISQ MKACDGINDC GDQSDCLCK ACQKGGFHCK 840
SGVCIPSQYQ	CNGEVDCTIG	EDEVGCAGFA SVTQEETEIL TADMDAERRR IKSLLPKLSC 900
GVKNRMHIRR	KRIVGGKRAQ	LGDLPWQVAI KDSAGITCGG IYIGGCWILT AAHCLRASKT 960
HRYQIWTTVV	DWIHPDLKRI	VIEYVDRIIF HENYNAGTYQ NDIALIEMKK DGNKKDCELP 1020
RSIPACVPWS	PYLPQPNDDC	IVSGWGREKD NERVFSLOWG EVKLISNCSK FYGNRFYEKE 1080
MECAGTYDGS	IDACKGDSGG	PLVCMDDANNV TYVWGVVSWG ENCGKPEFPG VYTKVANYPD 1140
WISYHVGRPF	ISQYNV	1156
SEQ ID NO: 22	moltype =	length =
SEQUENCE: 22		
000		
SEQ ID NO: 23	moltype = AA	length = 603
FEATURE	Location/Qualifiers	
source	1..603	
	mol_type =	protein
	organism =	Mus musculus
SEQUENCE: 23		
MKLAHLSLFL	LALHLSSSRS	PSASDLPQEE LVDQKCLLQK YTHRSCNKVF CQPWQRCIEG 60
TCICKLPYQC	PRAGTPVCAM	NGRSYPTYCH QKSFECLHPE IKFSHNGTCA AEGKFNVS LI 120
YGRKTGELV	QVKLVQDER	MFICKNSWSM AEANVACVDL GFPLGVVDIQ GSFNISGNLH 180
INDTECLHVV	CRGVETSLAE	CAFTKRRTTEL SNGLAGVV CY KQDADFPTSL SFQCVNGKHI 240
PQEKACNGVN	DCGQSDCLC	CKGCRGNASL CKSGVCIPDQ YKCNGEVD CI TGEDESRC EE 300
DRQQNIPKGL	ARSAQGEAEI	ETEETEMLTP GMDNERKRIK SLLPKLSCGV KRNTHTRRKR 360
VIGGKPANVG	DYPWQVAIKD	GQRITCGGIY IGGCWILTAA HCVRPSRAHS YQVWTALLDW 420
LKPNSQLGIQ	TVKRIVVHEK	YNGATFQNDI ALIEMKMHTG KKECELPNSV PACVPWSPYL 480
FQPNDRCIIS	GWGRGKDNQK	VYSLRWGEVD LIGNCSQFYP DRYYEKEMQC AGTRDGSIDA 540
CKGDSGGPLV	CEDINNVTYV	WGIVSWGENC GKPEFPGVYT RVANYFDWIS YHVGRSLVSQ 600
HNV		603
SEQ ID NO: 24	moltype = AA	length = 7
FEATURE	Location/Qualifiers	
source	1..7	
	mol_type =	protein
	organism =	Homo sapiens
SEQUENCE: 24		
MDANNVT		7
SEQ ID NO: 25	moltype = AA	length = 8
FEATURE	Location/Qualifiers	
source	1..8	
	mol_type =	protein
	organism =	Homo sapiens
SEQUENCE: 25		
PFISQYNV		8

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SEQ ID NO: 26	moltype = AA length = 10	
FEATURE	Location/Qualifiers	
source	1..10	
	mol_type = protein	
	organism = Homo sapiens	
SEQUENCE: 26		
REKDNERVFS		10
SEQ ID NO: 27	moltype = AA length = 12	
FEATURE	Location/Qualifiers	
source	1..12	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 27		
NTASSGADYP DE		12
SEQ ID NO: 28	moltype = AA length = 9	
FEATURE	Location/Qualifiers	
source	1..9	
	mol_type = protein	
	organism = Homo sapiens	
SEQUENCE: 28		
RPFISQYNV		9
SEQ ID NO: 29	moltype = AA length = 13	
FEATURE	Location/Qualifiers	
source	1..13	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 29		
GGGGGGGGGG GGG		13
SEQ ID NO: 30	moltype = AA length = 4	
FEATURE	Location/Qualifiers	
source	1..4	
	mol_type = protein	
	organism = Homo sapiens	
SEQUENCE: 30		
DGNK		4
SEQ ID NO: 31	moltype = AA length = 10	
FEATURE	Location/Qualifiers	
source	1..10	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 31		
CAGFASVAQE		10
SEQ ID NO: 32	moltype = AA length = 26	
FEATURE	Location/Qualifiers	
source	1..26	
	mol_type = protein	
	organism = Mus musculus	
SEQUENCE: 32		
CEEDRQQNIP KGLARSAQGE AEIETE		26
SEQ ID NO: 33	moltype = AA length = 6	
FEATURE	Location/Qualifiers	
source	1..6	
	mol_type = protein	
	organism = Homo sapiens	
SEQUENCE: 33		
KVTYTS		6
SEQ ID NO: 34	moltype = AA length = 9	
FEATURE	Location/Qualifiers	
source	1..9	
	mol_type = protein	
	organism = Mus musculus	
SEQUENCE: 34		
RSPSASDLP		9
SEQ ID NO: 35	moltype = AA length = 80	
FEATURE	Location/Qualifiers	
source	1..80	
	mol_type = protein	

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organism = synthetic construct
REPEAT      1..4
             note = single repeat of GGSS
VARIANT     1..80
             note = up to 19 copies of GGSS can be deleted
SEQUENCE: 35
GGSSGGSSGG SSGGSSGGSS GGSSGGSSGG SSGGSSGGSS GGSSGGSSGG SSGGSSGGSS 60
GGSSGGSSGG SSGGSSGGSS                                     80

SEQ ID NO: 36      moltype = AA length = 82
FEATURE           Location/Qualifiers
source           1..82
                 mol_type = protein
                 organism = synthetic construct
REPEAT          1..4
                 note = single repeat of GGSS
VARIANT         1..80
                 note = up to 19 copies of GGSS can be deleted
SEQUENCE: 36
GGSSGGSSGG SSGGSSGGSS GGSSGGSSGG SSGGSSGGSS GGSSGGSSGG SSGGSSGGSS 60
GGSSGGSSGG SSGGSSGGSS GG                                     82

SEQ ID NO: 37      moltype = AA length = 120
FEATURE           Location/Qualifiers
source           1..120
                 mol_type = protein
                 organism = synthetic construct
REPEAT          1..6
                 note = single repeat of GGSSGG
VARIANT         1..120
                 note = up to 19 copies of GGSSGG can be deleted
SEQUENCE: 37
GGSSGGGGSS GGGGSSGGGG SSGGGGSSGG GGSSGGGGSS GGGGSSGGGG SSGGGGSSGG 60
GGSSGGGGSS GGGGSSGGGG SSGGGGSSGG GGSSGGGGSS GGGGSSGGGG SSGGGGSSGG 120

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1. A formulation comprising a wild type complement factor I (CFI) or a variant thereof (CFI variant), wherein the formulation comprises a buffering agent, a surfactant, and a tonicity modifier.

2. The formulation of claim 1, wherein the formulation comprises one or more of a bulking agent, a cryoprotectant, and a lyoprotectant.

3. The formulation of claim 1, wherein the concentration of the CFI or variant thereof is present at about 10 mg/ml to about 300 mg/ml.

4. (canceled)

5. The formulation of claim 1, wherein the buffering agent comprises acetate, histidine, or succinate.

6. (canceled)

7. The formulation of claim 1, wherein the buffering agent is present at about 1 mM to about 50 mM.

8. The formulation of claim 1, wherein the surfactant is polysorbate 80, polysorbate 20, polysorbate 80/20, or poloxamer F68.

9. The formulation of claim 8, wherein the surfactant is present at about 0.001% to about 0.1% v/v.

10-11. (canceled)

12. The formulation of claim 1, wherein the tonicity modifier is sorbitol, trehalose, sodium chloride, or arginine hydrochloride.

13. The formulation of claim 1, wherein the bulking agent is trehalose or glycine.

14. The formulation of claim 1, wherein the tonicity modifier is selected from:

(i) sorbitol, wherein the sorbitol is present at about 1% to about 10% v/v;

(ii) trehalose, wherein the trehalose is present at about 1% to about 15% v/v;

(iii) sodium chloride, wherein the sodium chloride is present at about 1 mM to about 500 mM; and

(iv) arginine hydrochloride, wherein the arginine hydrochloride is present at about 10 mM to about 200 mM.

15-22. (canceled)

23. The formulation of claim 13, wherein the glycine is present at about 1% to about 5% v/v or about 1 mM to about 100 mM.

24-26. (canceled)

27. The formulation of claim 2, wherein the cryoprotectant or lyoprotectant is selected from glucose, sucrose, glycine, sorbitol, trehalose, sucrose, or mannitol.

28. (canceled)

29. The formulation of claim 28, wherein;

(a) the glucose is present at about 4% v/v;

(b) the sucrose is present at about 1% to about 10% v/v;

(c) the glycine is present at about 50 mM to about 150 mM;

(d) the trehalose is present at about 1% to about 10% v/v;

(e) the mannitol is present at about 1 mM to about 100 mM; or

(f) the sorbitol is present at about 1% to about 5% v/v.

30-41. (canceled)

42. The formulation of claim 1, wherein the formulation is selected from the formulations presented in Table 2.2 or Table 3.3.

43. The formulation of claim 1, wherein the formulation is in a solid form, a lyophilized form, or a liquid form.

44-45. (canceled)

46. The formulation of claim 1, wherein the formulation is stable at any one or more of -80°C. $+/-2^{\circ}\text{C.}$, -20°C.

C./-2° C., 0° C./-2° C., 4° C./-2° C., 25° C./-2° C., 37° C./-2° C., 45° C./-2° C., or at 60° C./-2° C.

47. The formulation of claim **1**, wherein the formulation is stable for at least 1 week, for at least one month, or at least one year.

48. The formulation of claim **1**, wherein the formulation comprises wild type CFI.

49. The formulation of claim **1**, wherein the formulation comprises a CFI variant comprising at least one modification with respect to a wild type CFI, wherein the CFI variant is capable of modulating the complement system, and wherein the CFI variant has at least one improved characteristic as compared to the wild type CFI.

50-53. (canceled)

54. The formulation of claim **49**, wherein the CFI variant is selected from those presented in Table 2.

55. The formulation of claim **1**, wherein the wild type CFI or CFI variant is a first component of a fusion construct

comprising a first component and at least a second component, and the wild type CFI or CFI variant is fused to the second component.

56-61. (canceled)

62. A formulation comprising a fusion construct comprising a wild type CFI or variant thereof (CFI variant), where the wild type CFI or CFI variant is fused to a human serum albumin, and the formulation is selected from those presented in Table 2.2 or Table 3.3.

63. The formulation of claim **62**, wherein the fusion construct comprises the amino acid sequence of SEQ ID NO: 21.

64. A method of treating a condition in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the formulation of claim **1**.

65-69. (canceled)

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